LUND UNIVERSITY

Soil salinity as a driver of microbial community structure and functioning

Rath, Kristin

2017

Soil salinity as a driver of microbial community structure and functioning

Soil salinity as a driver of microbial community structure and functioning

Kristin Rath

LUND
UNIVERSITY

Organization LUND UNIVERSITY Centre for Environmental Science and Climate Research (CEC) Microbial Ecology, Dept. of Biology Ecology Building, SE-223 62 Lund	Document name DOCTORAL DISSERTATION
	Date of issue: December 2017
Author: Kristin Rath	Sponsoring organization

Title and subtitle: Soil salinity as a driver of microbial community structure and functioning

Abstract

Soil salinization is a pressing agricultural problem in many areas of the world, particularly in areas heavily reliant on irrigation agriculture. While the negative effects of salinity on crop plants have been widely studied, its effects on soil microorganisms have received less attention, and the impact of soil salinity on both microbial community structure and functioning is not well understood. Soil microorganisms are important in regulating terrestrial biogeochemical processes and changes in microbial community structure and functioning in response to salinization could have far-reaching consequences for carbon and nutrient cycling.

The main aims of this thesis were to (a) assess and compare the impact of salinity on different microbial processes in soil, (b) study the ability of soil microbial communities to adapt to increasing salinity and connect increased tolerance to changes in community composition and functioning, and (c) study the combined impact of salinity and another important environmental disturbance, drought. The impact of salinity on soil microorganisms was studied at three different time scales: (i) acute salt exposure, (ii) exposure over one month and (iii) naturally saline soils having experienced high salt concentrations over long time periods.

I found that processes such as growth, respiration and N transformation rates were strongly negatively reduced in response to acute salt exposure. However, increased salt tolerance of the microbial community could be induced quickly, combined with a partial recovery of process rates. A strong relationship between soil salinity and community salt tolerance was also found along natural gradients of salinity. Higher community salt tolerance was reflected in shifts in the composition of the bacterial community. Fungi were less sensitive to salt exposure than bacteria, which manifested in less inhibition of fungal growth both after short-term and long-term exposure to salinity. When salinity was combined with drying of soils, the effects of the individual factors were exacerbated. Increased soil salinity also modified the microbial response to rewetting of died soil.

In summary, soil salinity was found to be an important regulator of both microbial functioning and community composition. While microbial communities have a strong potential to adapt to increasing salt concentrations which can offset to some defree the loss of functioning of the microbial community, process rates are still reduced in saline soils compared to non-saline soils. Thus, as salt-affected soils increase in area, the ability of microorganisms in saline soils to sustain biogeochemical cycles need to be considered.

Key words
Soil, salinity, microorganisms, fungi, bacteria, microbial growth, drying-rewetting, salt tolerance

Classification system and/or index terms (if any)

Supplementary bibliographical information		Language English
ISSN and key title		ISBN 978-91-7753-529-4 (print) 978-91-7753-530-0 (pdf)
Recipient's notes	Number of pages 166	Price
	Security classification	

4

Soil salinity as a driver of microbial community structure and functioning

Kristin Rath

LUND
UNIVERSITY

Table of Contents

List of papers

   I. Rath Kristin M and Rousk Johannes. Salt effects on the soil microbial decomposer community and their role in organic carbon cycling: A review. *Soil Biology and Biochemistry* 81 (2015), pp. 108-123

  II. Rath Kristin M, Maheshwari Arpita, Bengtson Per and Rousk Johannes. Comparative toxicities of salts on microbial processes in soil. *Applied and Environmental Microbiology* 82 (2016), pp. 2012-2020

 III. Rath Kristin M, Maheshwari Arpita and Rousk Johannes. Inducing community tolerance to salt in a microcosm experiment. *Manuscript*

 IV. Rath Kristin M, Fierer Noah, Murphy Daniel and Rousk Johannes. Using a trait-based approach to link bacterial community composition to salinity along soil salinity gradients. *Manuscript*

  V. Rath Kristin M, Murphy Daniel and Rousk Johannes. Bacterial and fungal biomass and growth rates along two gradients of soil salinity. *Manuscript*

 VI. Rath Kristin M, Maheshwari Arpita and Rousk Johannes. The impact of salinity on the microbial response to drying and rewetting in soil. Soil Biology and Biochemistry 108 (2017), pp. 17-26

Author contributions

I. KR wrote the first draft of the manuscript and JR contributed to the revision

II. KR, JR and AM planned the experiment. KR and AM collected and analysed data. JR and PB contributed to the interpretation of the data. KR wrote the first draft of the manuscript and all authors contributed to the revision

III. KR, JR and AM planned the experiment. KR and AM collected and analysed data and JR contributed to the interpretation. KR wrote the first draft of the manuscript and all authors contributed to the revision.

IV. JR planned the project and DM carried out sample collection. KR and JR planned experiments. KR and NF collected and analysed data, with all authors contributing to the interpretation. KR wrote the first draft of the MS, and all authors contributed to the revision.

V. JR planned the project and DM carried out sample collection. KR and JR planned experiments. KR collected and analysed data, with all authors contributing to the interpretation. KR wrote the first draft of the MS, and all authors contributed to the revision.

VI. KR, AM and JR planned the experiment, and KR and AM carried it out. KR analysed the data and JR contributed to the interpretation of the data. KR wrote the first draft of the manuscript and all authors contributed to the revision.

List of author abbreviations (initials)

KR = Kristin Rath
JR = Johannes Rousk
PB = Per Bengtson
AM = Arpita Maheshwari
NF = Noah Fierer
DM = Daniel Murphy

Popular Science Summary

High concentrations of salts in agricultural soils are an environmental problem that has plagued human civilization from its very beginning. Already the earliest civilizations in ancient Mesopotamia struggled with increasing concentrations of salt on irrigated fields. Also in modern times, salinization of soils, particularly of agricultural soils is still a problem restricting agricultural productivity in many areas. Irrigation can make areas suitable for agriculture that would otherwise be too dry to support high crop yields. However, water used for irrigation of fields evaporates and leaves behind dissolved salts, which over time accumulate in the soil to levels that negatively affect growth of crop plants. In order to achieve food security amidst growing population figures, agricultural productivity will have to be increased. Much of the projected population growth is going to take place in parts of the globe that experience low rainfall year-round or seasonally. Agriculture in these regions is heavily reliant on irrigation with water from groundwater or river sources, and many areas are already suffering from soil salinization. As irrigation agriculture intensifies the area of salt-affected soils will continue to increase.

For plants to be able to grow they need the support of huge numbers of microorganisms that live in soil. This group consists of, as the name suggests, small organisms that are difficult to see without a microscope. Bacteria form one major group of microorganisms. They are tiny single cell organisms that live in the small water-filled space between soil particles. Another major group of soil microorganisms are the fungi. They are well known for the often edible fruiting bodies that some of them produce, but below those fruiting bodies they form vast networks of filaments throughout the soil referred to as hyphae. A single gram of soil can contain billions of bacteria and kilometers of fungal hyphae. Bacteria and fungi are important as decomposers of dead organic material, such as leave litter and wood. During this process, microorganisms release nutrients bound in plant material that can be taken up by plants. Through their activity as decomposers microorganisms drive the biogeochemical cycling of carbon and nutrients in soil that is necessary to sustain life on earth. While we know much about the effects of soil salinization on plants, we know much less about its impact on microorganisms in soil and the processes carried out by them. Since the extent of salt-affected soils is increasing due to agricultural practices, it is important to understand the impact of salinity on soil microorganisms.

Broadly speaking, effects of environmental changes such as changes in the soil salinity on groups of organisms manifest in two ways. Firstly, they can influence which microorganisms can survive and thrive in the soil, and as a result determine the kind of species one can find at a specific site. This means, environmental change can influence the **structure** (or composition) of the community. Secondly, environmental conditions can change the microbial activity in the soil, and thus alter the rate at which they carry out all the important processes that microorganisms are responsible for. This means that the **functioning** of the community changes. These two properties of the community, **structure** and **functioning,** are highly interrelated and influence each other.

In this thesis, I studied the various stages in the response of the microbial community to increasing salt concentrations in experimental systems that experienced salinization at different time scales. Firstly I set up very short-term incubations of about one hour with salt added to soil, which covered the initial acute shock without adaptation by the microbial community. When salt is added to a soil, microorganisms will at first experience a shock in response to high salt concentrations. Many of them will die or become inactive, while only some will survive and continue to be active. This can be measured as a reduction in process rates in response to salt exposure. I was interested in how much different functions carried out by soil microorganisms are negatively affected by salinity and what that can tell us about which of these functions may be particularly sensitive to salinization. The processes that I measured included respiration, i.e. the release of CO_2 from the soil, which is a measure of the decomposer activity of microorganisms, and several processes involved in N cycling in soils. I also measured the rate at which fungi and bacteria produce new biomass, i.e. their growth rate. All of the processes were strongly inhibited by salt, showing that salinity can impede microbial functioning in soil. The short-term incubations revealed that bacteria are particularly sensitive to acute salt exposure, while fungi could maintain comparatively higher growth rates.

This first set of experiments only covered the immediate aftermath of salt exposure in the community. If given more time, after a short while those microorganisms that could survive the initial shock by salinity will undergo physiological changes that help them deal with the higher salinity. Not all microorganisms, however, have the same potential to adapt to the new salinity, and those species that can tolerate salinity better replace less well-adapted ones. Over time, this change in the species composition towards a community composed of more tolerant species increases the overall community tolerance to salt. To study the changes in microbial community structure and functioning during this adaptive phase, I gave microbial communities from a non-saline soil about one month to adapt to a range of new salinities in second set of experiments. What I was interested in was to see if and how quickly microbial communities can adapt

to increasing salinities, and how that increased salt tolerance is reflected in process rates. It turned out that microorganisms could adapt very quickly! Community tolerance increased within a week to a level matched to the soil salinity. At the same time growth rates, which were initially strongly reduced after salt exposure could recover to some degree, but in the end remained lower than process rates in non-saline soil.

How well communities can adapt to salinity is also limited by the pool of species present in the soil before salt addition. In a non-saline soil with no history of salt exposure, such as the one used for experiments 1 and 2 it is unlikely that very many of the species in that soil were able to adapt to high salinity. In places where salinity has had a long time to influence the community and select for salt-tolerant species, it could have resulted in highly adapted communities. The final study system therefore were soils coming from natural salinity gradients, where microbial communities had many years to adapt to the local salinity. Community tolerance strongly increased with salinity, and was accompanied by changes in the bacterial community structure, i.e. we found which bacterial species were salt-tolerant enough to withstand high salinities.

Salinity is not the only pressure that microorganisms in saline soils experience. Areas in which saline soils are common often undergo dry periods during which soils dry out. Drying also inhibits microbial activity in soils and in saline soils the combined impact of salinity and drying could make the effects of the individual factors worse. I tested this by drying soils of different salinity and found that indeed microorganisms were affected more strongly by drying if soils were also saline. Recovery after drying was also slower. After rewetting dried soils to the initial moisture levels, growth and respiration commenced immediately in less saline soils, while in saline soils a lag period of several hours occurred, during which respiration and growth were very low. These findings illustrate that combined environmental stresses can interact and exacerbate their individual effects.

In summary, my research shows that salinity has a strong inhibitory effect on the microbial community. Adaptation to salinity can offset to some degree the loss of functioning provided by the microbial community. This adaptation is accompanied by changes in the structure of the microbial community, i.e. shifts in which species are present in the soil. However, despite communities becoming more tolerant to salinity, some loss of functioning persists. As salt-affected soils are becoming an increasing problem in agriculture, the ability of microorganisms in saline soils to uphold important functions needs to be considered.

Acknowledgements

Johannes, it has been a joy to work with you on this project, and your enthusiasm for science is contagious. Throughout these four years your door has always been open, and you have always been available for immediate guidance when I felt stuck. I have been incredibly lucky to have you as the supervisor of my PhD thesis. You have been the best possible supervisor I could have had.

Per, in the end we did not work together very much, but I am nonetheless grateful for the help you provided, especially with using the ^{15}N pool dilution method.

Margarida, Lettice, Ainara, Eva, Carlos, and other past and present members of the Rousk lab, it was great to have been part of this awesome team. Thank you for the great discussions and good advice during lab meetings!

Saeed, I am grateful to have had you as an officemate for the past several years. Thank you for tolerating my messiness and grumpiness in the morning for all these years! It was great to be able to have all these interesting discussion with you and I wish you all the best for your own thesis!

Arpita, the outstanding work you did here at the department has greatly contributed to this project. Without you much less would have been possible.

Anna, for patiently teaching my how to perform qPCR. You have been a great help!

The microbial ecology group, thank you all for the warm welcome when I first arrived in Sweden, for the friendly atmosphere at work and for the laughs and great discussions over fika and lunch.

CEC, for the possibility to meet people from many different backgrounds and expand my horizon.

The Fierer lab, for hosting me and helping me with my data analysis. Special thanks to Tess!

Goffy, Kerstin, Lisa und Stenzi, ich bin so glücklich, dass unsere Freundschaften so lange gehalten haben. Ihr seid meine zweite Familie!

Gedis, you laughed when I said you would be acknowledged in this thesis, but your presence and support over all these years has been more important than you think. I am incredibly happy to have you in my life. Now I know that my decisions have turned out for the best, since they led me to you.

Mama und Papa, ohne eure Unterstützung wäre ich heute nicht, wo ich bin. Danke, dass ihr immer für mich da seid, auch über große Distanzen hinweg.

Introduction

Soil salinization

Soil salinization is one of the oldest manmade environmental problems. The Fertile Crescent, in ancient Mesopotamia, between the rivers Tigris and Euphrates, was made fertile by a network of irrigation channels delivering water from the rivers to the fields. The emergence of irrigation agriculture in Mesopotamia provided the basis for the development of the first complex urbanized societies in the area. The Sumerian city of Eridu, the oldest, or at least one of the oldest cities in the world, was built along a well developed system of irrigation channels. Eventually though, Eridu was abandoned around 2000 BC. Around the same time, many other Sumerian cities went into decline as well. Today, where there once was farmland, their ruins are surrounded by desert. Irrigation, the same technology that made the rise of civilization in Sumeria possible, had led to an accumulation of salts in the soil, drastically reducing agricultural output to the point were it could no longer support an advanced civilization (Perlin, 1989; Van de Noort, 2013).

Soil salinization poses serious environmental challenges not only in the ancient world, but also today. A soil is commonly defined as saline if the electrical conductivity measured in a saturated soil paste surpasses a value of 4 dS m^{-1} (Richards, 1954). However, plant growth can be negatively affected below that threshold. Globally, around 1 billion ha of land are to some degree affected by salinization (Szabolcs, 1989; Rengasamy, 2006) (**Fig. 1**). In Australia, a continent particularly severely affected by salinization, around 30% of the land area is classified as saline (Rengasamy, 2006). In Europe, soil salinization is a problem along the Mediterranean coastline (Daliakopoulos et al., 2016). Ca. 30% of the world's agricultural output is produced in irrigated cropland (Pitman and Läuchli, 2002), which is especially prone to salinization problems. About one third of irrigated cropland is estimated to be affected by salinization, reducing crop yields and posing a serious threat to sustainable food production (Pitman and Läuchli, 2002) (**Fig. 2**)
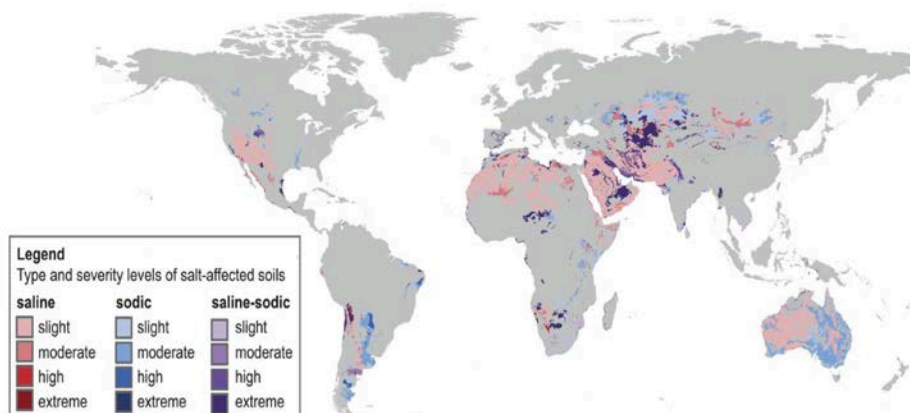
Fig. 1
Map of land areas that are affected to some degree by soil salinity. Reused with permission from Wicke et al. (2011), based on data from Fischer et al. (2008).

In 2000, the United Nations formulated eight international development goals, the Millenium Development Goals, which member states committed to achieve by the year 2015. As 2015 neared, plans developed to renew the Millenium Development Goals beyond the year 2015 in the form of the Sustainable Development Goals. These goals should set the foundation for a sustainable development on an economic, social and ecological level. One of those sustainable development goals is the commitment to eradicate world hunger by the year 2030. To meet this goal under the pressure of rising population figures, agricultural productivity will have to increase by 70% from current levels until the year 2050 (FAO, 2011). This amounts to 1 billion extra tons of cereals and 200 million extra tons of livestock products. Areas suitable for rain-fed agriculture are in most cases already under cultivation and at capacity (FAO, 2011). Much of that increase in agricultural output therefore will have to take place in areas reliant on irrigation agriculture in arid or semi-arid regions, or regions with a pronounced dry season. In many developing countries the quality of soils under irrigation is already suffering from unsustainable management practices, including the overuse of water resources and use of bad quality irrigation water.

Fig. 2
Salinized agricultural land in the Colorado River Basin, Utah, United States. Photo by Ron Nichols, USDA Natural Resources Conservation Service.

Naturally-occuring salinization of soil is often referred to as primary salinization. Naturally saline areas, such as salt lakes, salt pans and marshes are common in arid and semi-arid areas, as well as in coastal regions. Saline soils can occur naturally in areas with parent material rich in soluble salts or in areas with a shallow groundwater table that carries dissolved salts to the surface. Seawater intrusion can also lead to salinization problems in coastal areas. More problematically, the extent of salinized soils is increasing globally through several anthropogenic processes that lead to secondary salinization of soils. Two of the main mechanisms leading to secondary salinization include (i) irrigation-induced salinity and (ii) dryland salinity following land use change (**Fig.3**). In irrigation agriculture, poorly drained soils and the use of low quality irrigation water can over time lead to the accumulation of salts in the upper soil layer, as water evaporates and leaves salts behind. In dryland salinization, vegetation changes from deep-rooting tree species to shallow rooting crops have resulted in a rising groundwater table due to reduced evapotranspiration, mobilizing salts from deeper soil layers (Hatton et al., 2003). Additionally, vegetation changes in the opposite direction, from grassland to woodland have also on occasion resulted in rising salt concentrations of the soil, as low-lying saline groundwater becomes mobilized by deep-rooting trees (Jobbagy and Jackson, 2004).
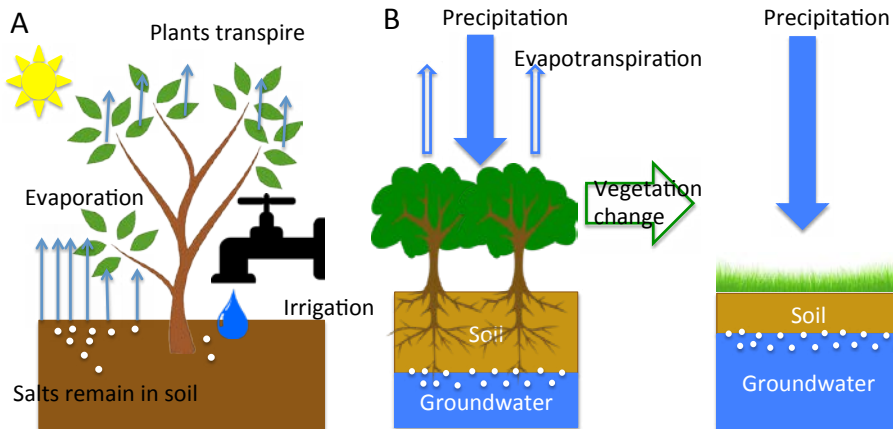
Fig.3
Schematic representation of two major mechanisms of secondary salinization: (A) irrigation salinization with evaporating irrigation water leaving behind dissolved salts in the soil, (B) dryland salinization with a vegetation change that resulted in decreased evapotranspiration leading to upward movement of the groundwater table, which carries dissolved salts with it.

Plants are negatively affected by soil salinity (see section below). As a result, plant growth in saline soils is stunted and agricultural crop yields are reduced. In non-agricultural systems slow-growing halophilic plants replace more productive species. Estimated annual costs of the loss in agricultural production caused by increasing soil salinity in irrigation agriculture amount to more than US$ 27 billion globally (Qadir et al., 2014). Estimates for India, a country strongly affected by soil salinization, reveal that farms operating in salt-affected areas suffer crop yield losses of about 50% due to salinity (Qadir et al., 2014).

Salt effects on living organisms

Cells are separated from the medium they live in by a cell membrane, which is permeable to water. When salt concentrations in the surrounding medium increase to a point where the solute concentration in the medium surpasses the solute concentration in the cell, cells loose water and run the risk of drying out, unless they counterbalance the increase in osmotic pressure. To survive at high salinity of their medium cells have to build up and maintain an equivalently high concentration of solutes in their cytoplasm. This is commonly achieved by the synthesis and accumulation of small organic molecules, called compatible solutes, because of their non-interference with cellular functions (Wood, 2011a). Some

compounds that are frequently used as compatible solutes include the sugar trehalose, amino acids such as proline and ectoine, and betaines (Kakumanu and Williams, 2014). Another strategy is to accumulate K^+ ions inside the cytoplasm, generally as a short-term response to rapidly increasing salt concentrations (Wood, 2011b). However, high ion concentrations can interfere with enzymatic processes, which is why most organisms prefer to synthesize small organic molecules. While compatible solutes are accumulated, salt ions that are toxic through their interference with intracellular enzymatic activities, such as Na^+ and Cl^-, must be excluded from cells. This exclusion happens through cross-membrane protein pumps. All of these adaptation mechanisms to high salt concentrations have a high energy requirement (Oren, 2011), making life in saline habitats energetically expensive.

The effect of salinity on organisms has been especially well studied in crop plants. Morphologically, the effects of soil salinity on plants manifest as stunted growth, necrosis of leaves, leaf yellowing and restricted root development (Bernstein, 1975). Plants suffer from high salt concentrations in a variety of ways (Hasegawa et al., 2000). Firstly, high intracellular salt concentrations are toxic to many cellular metabolic processes. For instance, more than 50 plant enzymes are activated by binding of K^+ ions. Na^+ can at high concentrations interfere with K^+ binding sites of intracellular enzymes, rendering them inoperable and disrupting metabolic processes (Tester and Davenport, 2003). High Na^+ concentrations thus result in necrosis of plant tissue and stunted growth. High Cl^- concentrations can induce chlorosis, i.e. a lack of chlorophyll, by degrading chlorophyll (Tavakkoli et al., 2010). Secondly, high salt concentrations in the soil limit the ability of plants to take up water into their root systems against a highly negative soil water potential. Finally, high salt concentrations can also lead to imbalances in the uptake of cations important for plant nutrition. While the effects of salt exposure on crop plants have been extensively studied, the impact of salinity on microorganisms has received less attention, despite them playing a key role in maintaining soil fertility.

The soil habitat

Soil refers to the upper layer consisting of a mixture of organic and mineral constituents that covers most terrestrial surfaces. This layer is the foundation of almost all of terrestrial life. Soil is a vital component of all global biogeochemical cycles, which move carbon (C), nitrogen (N) and other nutrients through various biotic and abiotic compartments and together form the basis for sustainable life on earth. At the same time, soil is one of the most complex and heterogeneous

habitats on earth. Particles and aggregates offer a variety of microhabitats with greatly varying conditions. This heterogeneous environment harbors an immensely numerous and diverse community of microorganisms, who through their activity as decomposers of organic material are key players in the terrestrial C and nutrient cycles. The C stored in soil organic matter comprises about 2500 gigatons (Gt) (Lal, 2004) and thus represents one of the largest active C pools in the global C cycle (**Fig. 4**). Major fluxes between the soil C pool and the atmospheric C pool include the input of plant-synthesized organic C compounds into soil and the efflux of C released from organic material by decomposer activity. The emerging view is that stabilized C in soil is not directly plant-derived, but is C that has passed through microbial biomass (Schmidt et al., 2011; Liang et al., 2017). Microbially-derived compounds can become stabilized through association with soil minerals and persist in soils for long time periods in organo-mineral complexes (Sollins et al., 2009; Liang et al., 2017). Changes in the relationship between C entering new microbial biomass and C released during breakdown of organic matter, i.e. the carbon use efficiency (CUE), could have important implications for the size of the terrestrial and the atmospheric C pools (Schlesinger and Andrews, 2000).

Decomposing microorganisms in soil can be broadly divided into two major groups: prokaryotic organisms (bacteria and archaea) on the one hand, and fungi on the other hand. Prokaryote decomposer organisms are generally unicellular organisms living in the water-filled pore-space between soil particles (**Fig. 5**). While a variety of different metabolic strategies can be found among soil bacteria, most soil bacteria utilize C compounds as their energy source. In contrast, fungi form vast hyphal networks throughout the soil. Some fungi are plant symbionts that live in a symbiotic association with plant roots called mycorrhizae. However, this thesis focuses on the saprotrophic fungi, a group of non-symbiotic fungi breaking down dead organic material. One gram of soil can contain up to 10^{10}-10^{11} bacterial cells and several km of fungal hyphae. Generally microbial decomposers break down organic material in the soil by excreting extracellular enzymes into the soil environment, which break down larger polymeric molecules into smaller compounds that can be taken up into to the cells. Fungi are able to produce a wider array of extracellular enzymes than bacteria and are thus typically able to decompose more complex organic substrates (de Boer et al., 2005; Romani et al., 2006; Strickland and Rousk, 2010).
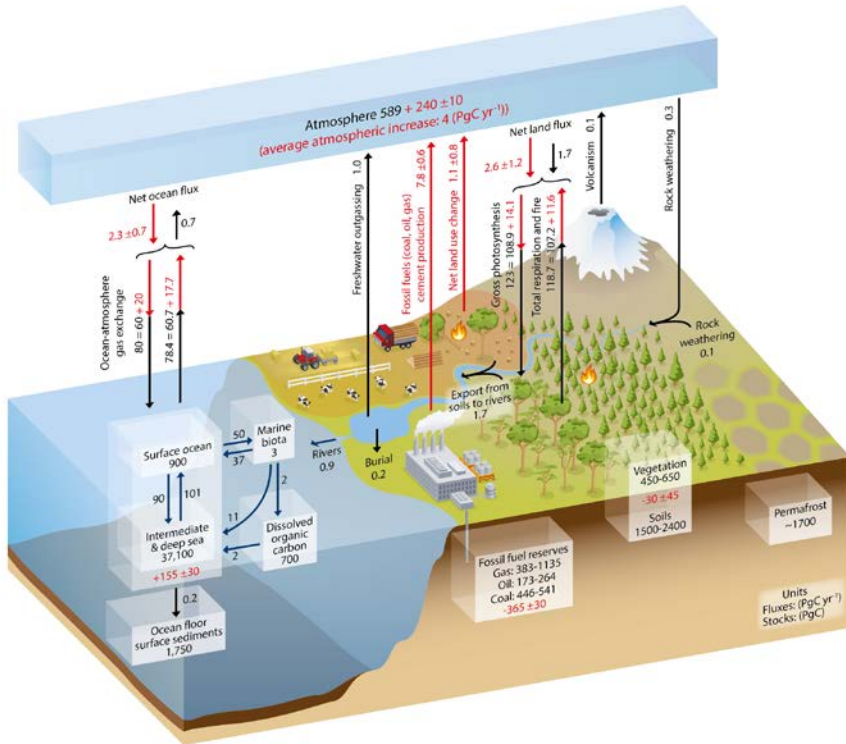
Fig. 4
Schematic representation of the global carbon cycle. The numbers represent estimated carbon stocks in PgC and annual C fluxes in PgC yr^{-1}. Black numbers represent estimates of pools and fluxes before the beginning of the Industrial Era, while red numbers represent anthropogenic changes since the beginning of the Industrial Era ca 1750. Image used from Ciais et al. (2013).

Microorganisms are also of great importance in the cycling of elements other than C. Nitrogen (N) is one of the most important nutrients for plant growth and its availability often limits plant productivity, i.e. plant biomass can only increase if more N is made available to them. During microbial decomposition of organic material N and other nutrients bound in dead organic matter are released as ammonium (NH_4^+) and made available for uptake by both microorganisms and plants. By recycling nutrients essential for plant growth, soil microorganisms are vital for the maintenance of soil fertility. At the same time, microorganisms can also contribute to N loss from the system, by converting NH_4^+ to nitrate (NO_3^-), which is more easily leached from soil, or by further reducing NO_3^- to gaseous N compounds. Thus microorganisms through their activity determine the turnover rate of N, as well as its residence time in soil.
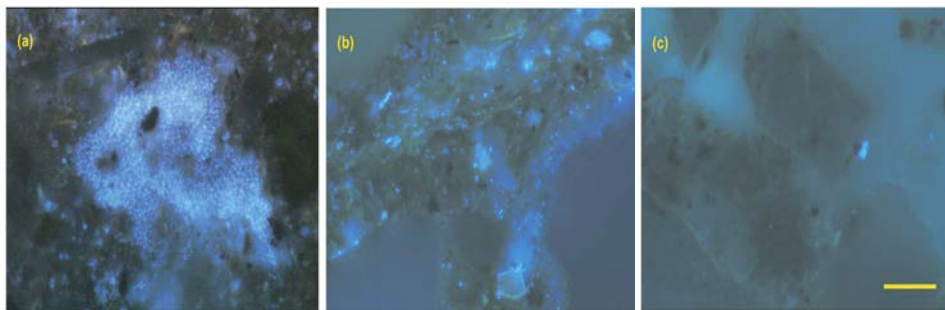
Fig. 5
Bacterial cells in soil thin sections (a) after the addition of glucose, (b) after harvest and (c) in subsoil. The scale bar corresponds to 20 µm. Image used with permission from Nunan et al. (2003).

Microbial decomposer activity is not only regulated by the availability of organic substrate but also by a variety of biotic and abiotic environmental factors. One such factor often recognized as a determinant of microbiological process rates is temperature (Lloyd and Taylor, 1994; Kirschbaum, 1995; Fierer et al., 2006); another one is the availability of water, which also provides the medium through which the accessibility of substrate for decomposition is regulated (Schimel et al., 1999; Manzoni et al., 2012). Salinity is another abiotic factor that can strongly decrease microbial activity (Setia et al., 2011; Rath and Rousk, 2015), but its effect on microbial community structure and function is not well understood, and has received limited attention to date.

The effect that salinity has on plants and microorganisms has potential impacts on the soil organic matter contents of soils through several interacting mechanisms. Reduced plant growth is reflected in lowered input of new organic matter into saline soils. At the same time, decreased microbial activity could lower the decomposition rate and increase the turnover time of C in saline soils. However, since microbial necromass is the origin of the most stable C fractions in soil (Schmidt et al., 2011; Liang et al., 2017), a decreased synthesis of new microbial biomass could lead to a lower rate of stabilization of C (**Fig. 6**). The cost of adaptation to salinity could result in lower carbon use efficiency with higher respiration per unity of new biomass. The interaction between these several mechanisms is critical to understand in order to predict whether soil salinization will result in a decrease in soil C stocks (Setia et al., 2013).
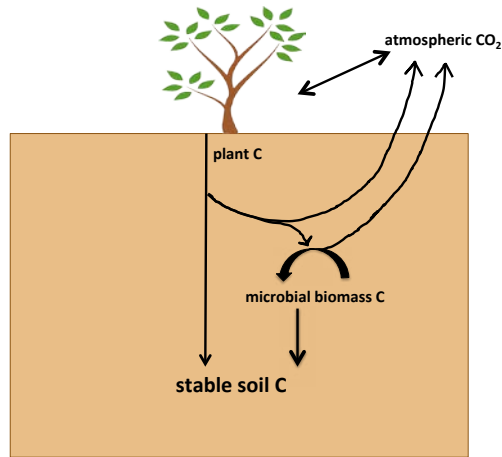
Fig.6
Schematic representation of the flow of C from plants to the stable soil C pool. Most stabilized C in soil has passed through the microbial biomass. The relationship between incorporation of C into microbial biomass and release as CO_2 is important in determining the role of soil as a C source or sink.

Salinity effects on fungi and bacteria

Generally, highly saline habitats are dominated by prokaryotic organisms. In saline soils, shifts towards a more bacteria-dominated community with increasing salinity have been reported (Pankhurst et al., 2001; Chowdhury et al., 2011). Similarly, communities in aquatic hypersaline habitats such as salt lakes or solar salterns contain mostly prokaryotes (Grant, 2004). This has lead to the widespread view that fungi are more susceptible to high salt concentrations and that soil salinity favors bacterial decomposers (Pankhurst et al., 2001). However, it is not clear why fungi should be more sensitive to high salt concentrations in soils. Fungi can be extremely tolerant of low water potentials. In environments where osmotic pressure is caused by high concentrations of organic solutes such as sugar-preserved foodstuffs, filamentous fungi and yeasts dominate (Grant, 2004). Fungal growth in high-sugar foodstuffs can occur even at the lowest water potentials at which life has been recorded (Grant, 2004). Moreover, fungi have been found to be more resistant than bacteria to exposure to heavy metals (Rajapaksha et al., 2004), which are also a type of salt and could thus have a similar mode of toxic action on microorganisms. However, naturally saline habitats frequently have a high pH, which favors bacteria over fungi (Rousk et al., 2010). It is possible, that the lower relative abundance of fungi in saline habitats is a consequence of the

high pH in these habitats, rather than a direct effect of high salinity (Wichern et al., 2006; Kamble et al., 2014).

Shifts in the fungi-bacteria ratio in response to environmental changes have implications for C dynamics in the soil. Generally, microbial necromass is the origin of stabilized and stored C in soil (Liang et al., 2017). Fungal residues are less easily decomposable and have a slower turnover rate in the soil environment than bacterial residues (Guggenberger et al., 1999; Six et al., 2006; Rousk and Bååth, 2007b). The fungi-bacteria ratio could also have implications for the CUE of soils (Strickland and Rousk, 2010). Thus, shifts in the ratio of these two groups in response to salinity could change the residence time of C in soil and affect its C sequestration potential.

Salinity and drought

Saline soils can mainly be found in arid and semi-arid regions of the globe, where high salt concentrations and drought pose coinciding constraints on microbial activity. High salt concentrations and low water contents of soils have a similar mode of action, by limiting the availability of water to soil microorganisms. Consequently, adaptation mechanisms are thought to be similar, with organisms synthesizing compatible osmolytes to counteract both high osmotic pressure and low water availability (Kakumanu and Williams, 2014). Both high salinity and low soil moisture on their own have negative effects on soil microorganisms (Mavi and Marschner, 2012), but combined these two factors have the potential to exacerbate their individual effects.

Microorganisms in soil are exposed to fluctuating water levels. Commonly, rewetting dry soils results in a high respiration pulse immediately following rewetting (Birch, 1958). The mechanisms behind this respiration pulse are not entirely clear, and several explanations have been put forward, ranging from physical mechanisms, such as the release of gases from soil spore space, to biochemical, such as the activity of enzymes that break down newly availably substrate. In any case, the respiration pulse is uncoupled from the microbial growth response (Meisner et al., 2013). The responses of bacterial growth and respiration to drying-rewetting usually follow one of two general patterns (**Fig. 7**). In a type 1 response, upon rewetting bacterial growth either increases linearly starting immediately after rewetting, accompanied by one immediate respiration pulse followed by a steady decrease in respiration over time. In a type 2 response a lag-phase of no growth occurs, followed by an exponential increase in growth,

while respiration rates are elevated immediately after rewetting, occasionally followed by a secondary increase in respiration (**Error! Reference source not found.**) (Meisner et al., 2013; Meisner et al., 2015). Whether or not a lag phase in growth occurs, has been proposed to be influenced by the severity of drying experienced by the microbial community. Since salinity increases the severity of drying it is expected to influence the microbial response to drying-rewetting, with implications for C dynamics in saline soils subjected to fluctuating water contents.
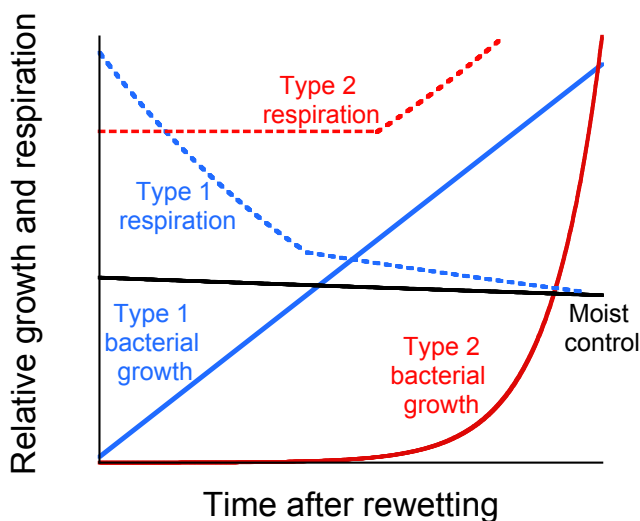
Fig. 7
Schematic representation of a type 1 (blue) and type 2 (red) response of bacterial growth (solid line) and respiration (dashed line) to rewetting of dried soil.

The study systems

This thesis includes studies of the effect of salinity on three different time scales: (i) short-term assessments of the effects of acute salt exposure over a period of a few hours, (ii) microcosm experiments over a period of one to two months, (iii) natural salinity gradients having experienced high salt concentrations for centuries. Using three different time scales allowed me to study different aspects of the effect of salinity on soil microbial communities. When microbial communities are exposed to salinity three different mechanisms can lead to adaptation of the community: (i) physiological changes of individual cells, e.g. by inducing the synthesis of osmolytes, (ii) changes in the composition of the community through species sorting, i.e. more salt-tolerant species replace less salt-tolerant ones and

(iii) evolutionary changes, through selection of tolerant genotypes within a population (Blanck and Wängberg, 1988). The relative importance of these adaptation strategies varies over time scales, with physiological adaptions dominating in short-term responses, whereas evolutionary changes and shifts in the community composition are a consequence of long term exposure to salt.

Short-term assays in soils with no history of salt exposure that proceed over a few hours do not give the microbial community enough time to adapt to the salt exposure. They can be used to compare the potential inhibition of various microbial processes by salinity without being confounded by the potential of various functional groups of decomposers to adapt to high salinity. Short-term assays can also be used to compare the impact of different salts on microbial processes, since communities should not have prior tolerance to any of the salts used. On the other hand, short-term assays do not provide insight on the resilience of microbial processes, that is their ability to recover from disturbance, and thus cannot predict the long-term impact of salinity.

Microcosm experiments that continue over a period of weeks to months allow us to study the potential of communities to adapt to changed salt concentrations, both through physiological adaptions and shifts in community composition, and compare the resilience of different microbial processes. Another advantage of laboratory microcosm studies is that other factors than salinity can be kept at constant levels, allowing for isolation of the salt effect from other factors. A disadvantage of microcosm studies is that laboratory systems are difficult to scale up to field situations. Moreover, the communities are limited by the starting species pool present in the soil used for the experiment, without potentially better adapted species coming in through dispersal (Berga et al., 2017).

Finally, the impact of long-term exposure to salinity on microbial community structure and function can be studied along natural gradients of salinity, where a long history of salinity has filtered for a community composed of salt-adapted species, together with evolutionary changes leading to the development of more salt-tolerant strains. Environmental gradients are difficult study systems, since environmental factors are usually highly auto-correlated. This makes it difficult to establish with certainty a causative relationship between a specific environmental factor and any observed effect on the microbial community. For instance, high salt concentrations usually co-vary with organic matter contents of soil and the type of plant community present at a site.

The natural salinity gradients used in this thesis are two gradients located at a salt lake in Western Australia (32°29' S, 119°12' E), adjacent to Lake Liddelow Nature Reserve (**Fig. 8**). Each gradient was sampled in three transects with 8

sampling points per transect, beginning from the lakeshore. In total 24 samples were collected for each gradient. One gradient was located at the north shore of the lake, with sampling points 5-8 at the less saline end of the gradient located in agricultural land. The other gradient was located approximately 2.5 km across the lake on the south shore and extended into eucalyptus forest, the natural climax vegetation of the area. Salt crusts covered the sites close to the lakeshore at both gradients, with sparse vegetation consisting of halophilic plant species. Salinity along the gradients ranged from about 0.1 dS m^{-1} to 100 dS m^{-1} in a saturated paste, and thus includes both non-saline and highly saline soils. This salinity covers the same range of salinity as going from a freshwater lake to a salt lake.

Fig. 8
The shore of Lake Conner, the salt lake studied in this thesis. Picture by Andrew Wherrett.

Methods to assess the status of the microbial community

Microbial biomass, growth and respiration

One of the most frequently assessed parameters in studies of environmental impacts on microorganisms is the size of the microbial biomass (Rath and Rousk, 2015), with a variety of methods developed for this purpose, of which I will here introduce some of the most important ones. One of the most common methods used to measure biomass is chloroform-fumigation-extraction (CFE). In this method, soils are fumigated with chloroform to break up cells and release the labile C contained in them. Then C is extracted from the soil, and fumigated samples are compared to non-fumigated controls. The difference in extracted C between fumigated and non-fumigated samples is converted into a measure of microbial biomass (Joergensen, 1996). Another commonly used method that specifically targets living microbial biomass is substrate-induced respiration (SIR) (Anderson and Domsch, 1978). Soil is supplied with glucose and after a short incubation period of one to two hours, respiration is measured. The respiration pulse following glucose addition should be proportional to the amount of microbial biomass in the soil. Many other methods rely on measuring the concentration of specific biomarker substances and then convert these measurements into estimations of biomass size. For instance, one commonly used method is analysis of the phospholipid fatty acid (PLFA) content of soil. PLFAs are key components of microbial cell membranes and can be converted to microbial biomass using the average PLFA concentration per cell (Frostegård and Bååth, 1996). Since taxonomic groups differ in their PLFA profile, PLFAs can also be used to describe the composition of the microbial community on a broad taxonomic level. Ergosterol, a lipid found in the cell membranes of fungi, is another such biomarker that is used to specifically measure the fungal biomass of soil (Montgomery et al., 2000).

The underlying assumptions of biomass measurements are that impacts of environmental changes will be reflected in changes of the microbial biomass and that the size of the microbial community corresponds to the rates at which microbial processes are carried out. However, during any given period, the vast majority of the microbial biomass in soil is of unknown status, with the proportion of active microorganisms generally making up only $0.1 - 2\%$ of the total microbial biomass (Blagodatskaya and Kuzyakov, 2013). This makes biomass a poor predictor of actual process rates (Rousk, 2016). In addition, microbial biomass levels do not respond quickly to changes in environmental conditions, since

biomass levels are a function of many different processes, such as growth, death, predation and turnover of biomass.

Process rates are a more direct and sensitive way of measuring the impact of environmental changes on the microbial community. Processes can be related to catabolism, i.e. the breakdown of organic substrate to produce energy, or anabolism, the synthesis of new biomass. The rate at which new biomass is produced, or the growth rate, can be measured by tracing the incorporation of labeled precursor molecules into new biomass. For instance, bacterial growth can be measured by measuring the rate of incorporation of radio-isotopically labeled leucine, an amino acid, into bacterial protein in a soil suspension, following a homogenization-centrifugation procedure (Bååth et al., 2001). Following the same principle, fungal growth can be measured as the incorporation of radio-isotopically labeled acetate into ergosterol (Bååth, 2001). Other approaches measure microbial growth through the uptake of ^{18}O-labelled H_2O into microbial DNA (Spohn et al., 2016), or use ^{13}C-labelled metabolic tracers, such as glucose or pyruvate (Hagerty et al., 2014).

The activity of decomposing microorganisms is often measured as respiration, i.e. the release of CO_2 from soil. CO_2 is the end-product of energy production in aerobic heterotrophic microorganisms. How much of organic substrate is used by microorganisms to build new biomass and how much of it is released as CO_2, the so called carbon use efficiency (CUE), determines whether soil serves as a source or sink for C. Environmental disturbances and stress are some of the factors thought to shift CUE towards higher release via respiration and thus C loss from soil (Wardle and Ghani, 1995; Manzoni et al., 2012). As adaptation mechanisms to high salt concentrations are energetically expensive, a shift towards lower CUE is expected.

Microbial communities

Some of the biomarker-based methods to measure biomass can also yield crude information on what types of microorganisms are present in a soil. Much more detailed information on the composition of the soil microbial community is available from molecular biology techniques. The most common way to characterize microbial community composition is to use amplicon sequencing, usually of regions of ribosomal rRNA genes, followed by a comparison of the obtained DNA sequences to available sequences in databases.

It can be difficult to interpret the vast amounts of information provided by molecular analyses of community compositions in a meaningful way. Microbial community composition can often be correlated with environmental variables. One issue when establishing relationships between the abundance of certain taxa and the environment based on correlation is the auto-correlation of environmental variables, making it difficult to disentangle the effect of one factor on the microbial community from other factors. Another problem of microbial community composition assessments is that abundances of taxa are difficult to quantify and therefore usually only assessed as relative abundances. A taxon can therefore appear to respond positively to an environmental factor by increasing in relative abundance, despite its absolute abundance remaining unchanged, simply because other taxa have decreased. Functional redundancy of microbial communities is thought to be high, meaning that different taxa are capable of carrying out the same functions in the environment (Strickland et al., 2009). However, relationships between community structure and function have been identified (Reed and Martiny, 2007; Strickland et al., 2009; McGuire and Treseder, 2010), which means that community composition can modify the rates at which processes are carried out. Understanding how the environment drives the composition of the microbial community is therefore important for a predictive understanding of ecosystem functioning.

Molecular techniques can also be used to estimate the absolute abundance of certain genes in a soil. For example, by quantifying the abundances of bacterial 16S rRNA genes and fungal 18S rRNA genes the absolute abundance of fungi and bacteria in soil can be estimated. A method commonly used for this purpose is quantitative polymerase chain reaction (qPCR). This method selectively amplifies certain sections of the gene of interest and measures the increase of DNA after each PCR cycle. The initial quantities of DNA in soil can be estimated, because the rate of increase in the amount of DNA during PCR is proportional to the starting quantities of the target gene.
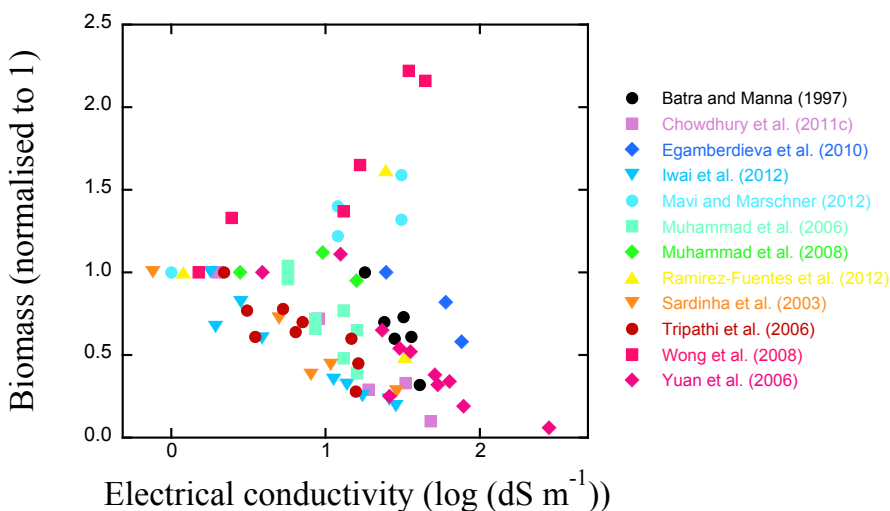
Fig.9
Compilation of biomass measurements from published studies. Biomass measurements have been normalized to the value of the lowest salinity sample included in each study. Figure from paper I.

Main results and conclusions

Salinity inhibits microbial processes more so than biomass

The most commonly analyzed parameters of soil microbial responses to salinization are changes in microbial biomass and respiration. In a compilation of data from a wide range of published studies, it emerged that overall respiration is negatively affected by salinity, consistently in both short- to medium-term laboratory studies and along natural salinity gradients (Paper I) (**Fig. 9**). At the same time microbial biomass in relation to organic C contents of soil showed no consistent trend with soil salinity, neither following long- nor short-term exposure to salinity (**Fig. 10**). This suggests that microbial biomass is not a sensitive or responsive parameter to evaluate the impact of environmental disturbance on the microbial community. Considering that biomass is a function of several processes, including growth rate, mortality and turnover, changes in any one of these processes will only slowly manifest as a change at the total biomass level. Microbial biomass also appeared to be only indirectly related to active process rates in soil (Paper I, III).
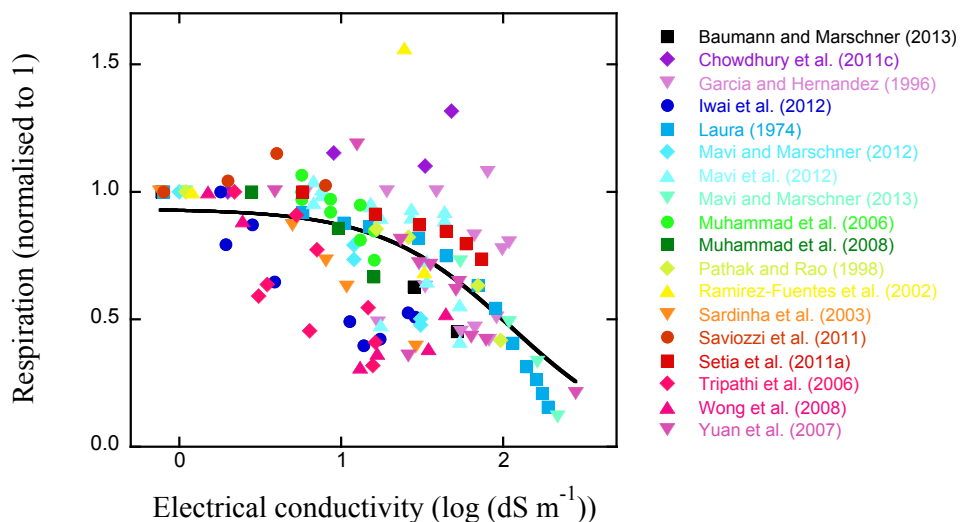
Fig. 10
Compilation of respiration measurements from published studies. Respiration measurements have been normalized to the value of the lowest salinity sample included in each study. Figure from paper I.

Differential effects of salinity on bacteria and fungi

It has generally been assumed that fungi are less resistant to salinity than prokaryotes, based on the prokaryotic dominance in many hypersaline habitats (Gunde-Cimerman et al., 2000). Similarily, saline soils are often thought to be dominated by prokaryotic decomposers (Pankhurst et al., 2001; Rath and Rousk, 2015). However, in a comparison of salt toxicity on different microbial processes it emerged that fungal growth was more resistant to acute salt exposure than bacterial growth (Paper II) (**Fig. 11**).
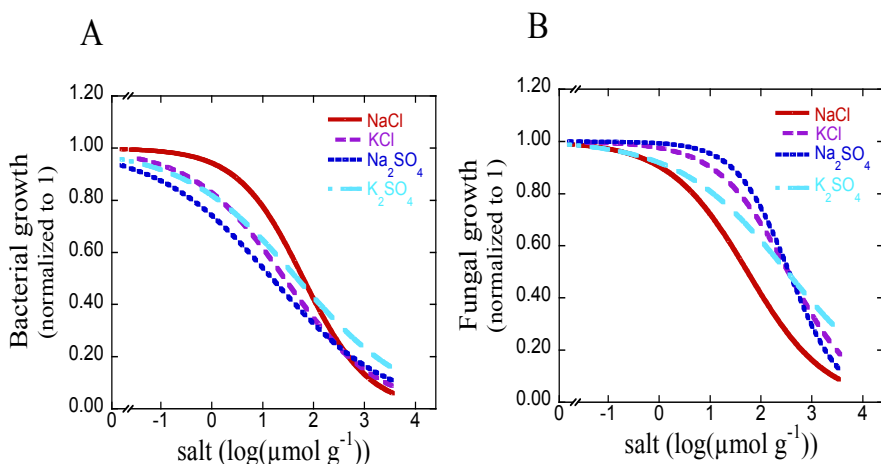
Fig. 11
Dose-response relationships between (A) bacterial growth and (B) fungal growth and short-term exposure to different salts (NaCl, KCl, Na$_2$SO$_4$ and K$_2$SO$_4$).Error bars indicate the standard error (n=2) (Paper II).

Several mechanisms could have contributed to higher fungal salt tolerance. Firstly, the chitinous cell wall of fungi increases their protection against low water availability (Strickland and Rousk, 2010), which could also make them more resistant to osmotic pressure. Secondly, the intracellular localization of the proton gradient used for energy generation in eukaryotes could make them more resistant to high external cation concentrations compared to prokaryotes, who have to maintain this gradient across their external membranes. Fungi have also been found to be more resistant to heavy metal exposure (Rajapaksha et al., 2004), and the mechanisms for higher heavy metal tolerance could be similar. Over time-scales of weeks to months in a microcosm experiment, fungi were better able to recover after exposure to high salinities than bacteria (Paper III). Consistent with higher fungal resistance to short-term salt exposure, along natural gradients of long-term salt exposure fungal growth was also less impacted by salinity than bacterial growth (Paper V) (**Fig. 12**).
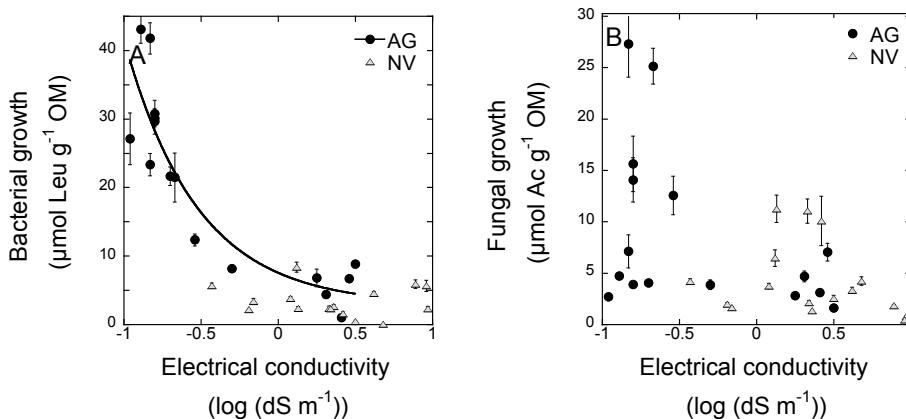
Fig. 12
Cumulative bacterial growth and fungal growth over 17 days along two natural salinity gradients (Paper V).

Using trait-based approaches to connect salinity to community composition

Despite a large amount of data generated on microbial community composition in different environments, a basis for predicting changes in community composition in response to environmental change has remained elusive. The development of trait-based approaches has been proposed as a promising way forward (Webb et al., 2010; Wallenstein and Hall, 2012). Trait-based approaches look at microorganisms in terms of their biological properties. Functional traits are properties of microorganisms that strongly impact their performance and ability to survive under certain conditions. Webb et al. (2010) put forward a conceptual framework for using trait-based theory consisting of three elements: (i) trait distributions describing the frequency with which values of a functional trait occur in the community; (ii) the local environment that serves as performance filters on the distribution of functional traits by favouring traits that provide high fitness in the local environment and selecting against those that do not; and (iii) environmental gradients along which the performance filter is projected.

The natural salinity gradients used in this thesis provide a suitable system to test the use of a trait-based approach to study microbial community composition. Salinity along the gradients served as a performance filter for the functional trait of salt tolerance. At sites of high salinity, the local environment would have selected for microorganisms with high salt tolerance, shifting the trait distribution of salt tolerance towards higher frequency of high salt tolerance, whereas at low salinity sites the trait distribution of salt tolerance was unconstrained. Unfortunately, in microbial ecology values of functional traits are difficult or even

impossible to assign to individual members of a community (Green et al., 2008). A way around this is to estimate tolerance trait distributions aggregated at the community level. For instance, salt tolerance can be measured by establishing inhibition curves for bacterial growth in response to salt addition. A community that, due to past salt exposure, has its trait distribution shifted towards higher frequency of high salt tolerance, should be more resistant to salt additions than a community without a legacy of salt exposure.

At the community level, one of the ways filtered trait distributions manifest is as changes in community composition. Measuring the extent to which exposure to an environmental factor has shifted functional trait distributions allows for the identification of important drivers of community composition. Only factors that have constrained community composition to the extent that they have resulted in filtered trait distributions play an ecologically relevant role in controlling microbial community composition.

In communities with a legacy of salt exposure salinity would have selected for members with a high salt tolerance and disfavored salt-sensitive species. This selection for high salt tolerance resulted in a filtered trait distribution of salt tolerance (Paper IV) (**Fig. 13**). Changes in the trait distribution of salt tolerance were matched to the local environment, as evidenced by a strong correlation between soil salinity and community salt tolerance, and could be connected to shifts in the community composition. Consistent shifts in community composition in response to high salinity allowed for the identification of biomarker species for salt tolerance (Paper IV). This trait-based approach has rarely been applied to microbial communities and is one of the first comprehensive assessments of microbial community composition along a wide range of soil salinities.

While processes are strongly inhibited in response to acute salt exposure in non-saline soil (Paper I), this inhibition can partially be offset in saline soils (Paper IV, Paper V). In naturally saline soils microbial activity could still be measured at salt concentrations at which it was almost completely inhibited in short-term assays. This indicates that shifts in community salt tolerance help to maintain biogeochemical processes under conditions at which they would otherwise be inhibited. Microbial communities are quickly able to respond to high salinities by adjusting their tolerance to salt within days to weeks (Paper III).
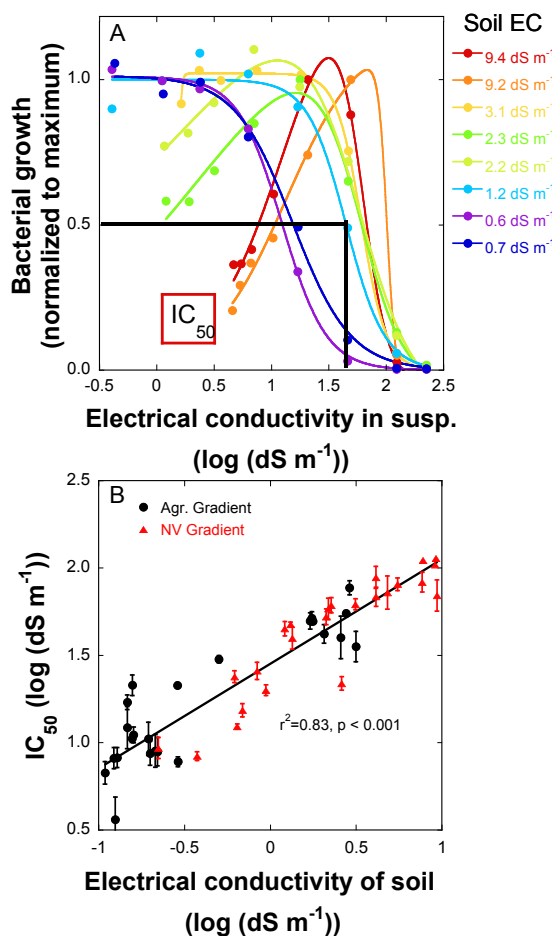
Fig. 13
Left panel: Relationship between community salt tolerance and soil salinity along two environmental salinity gradients. Right panel: Dose-response relationships between bacterial growth (normalised to level in control) and salt exposure in samples with different salinities (salinities denoted in figure legend). (Paper IV).

Organic matter additions alleviate process inhibitions by salt

Adaptation mechanisms by the soil microbial community to salinity are energetically expensive (Oren, 1999). Synthesis of compatible solutes, usually small molecular weight C compounds, also requires availability of C substrate to microorganisms. However, organic matter content in saline soils is often low (Wong et al., 2010), restricting the ability of microorganisms to acquire the needed substrate. Some of the negative effects of salinity on microbial processes in saline

soils could therefore be caused by low substrate availability rather than by salinity directly. Organic amendments of soils are frequently used remediation strategies in saline soils (Tejada et al., 2006). In addition to the positive effects of organic amendments on soil structure of saline soils, they have also been found to increase microbial biomass and activity (Tejada et al., 2006). By supplying the microbial community with additional organic substrate, substrate limitation in saline soils can be alleviated and indirect effects of the low substrate availability can be distinguished from other salt effects. In paper V soils from a natural salinity gradient were supplied with plant material to overcome low organic matter availability. Inhibition of processes by salinity decreased with higher availability of organic material, indicating that some of the reduction of microbial process rates in saline soils was caused by low organic matter availability. This result highlights the potential for organic matter additions as a remediation measure to improve soil fertility in saline soils. However, microbial growth and activity remained lower in saline soils than non-saline soils, suggesting other effects of salt played a role as well in reducing microbial processes.

Salinity modifies the microbial drying-rewetting response by increasing harshness of drying

Salinity increased the severity of drying experienced by the microbial community (paper VI). This was evidenced by the fact that during air-drying of soils of different salinities, bacterial growth and respiration rates at the same water content were more strongly inhibited in saline soils compared to non-saline soil. As discussed in the introduction section, the severity of drying has a strong impact on the response of the microbial community to rewetting of dried soil. Since salinity increased drying severity, we expected the drying-rewetting response in saline soil to change in a predictable manner. Consistent with this expectation, we found that non-saline soils or soils with low salinity responded to drying-rewetting with a typical type I response (**Fig. 7**), i.e. an immediate respiration pulse accompanied by a linear increase of growth after rewetting (**Fig. 14**). Saline soils on the other hand showed a prolonged lag period in growth, followed by an exponential increase, consistent with a type II response of growth. Respiration, however, did not follow a typical type II response, since respiration rates were initially reduced below the level observed in a continuously moist control soil, and only increased following a lag period (**Fig. 14**). This delayed respiration response in saline soils could elucidate some of the mechanisms behind the respiration pulse observed after rewetting, since it indicates that salinity has an inhibiting effect on those mechanisms. The different drying-rewetting responses of saline and non-saline soils could have implications for C dynamics in these soils during fluctuating moisture conditions.
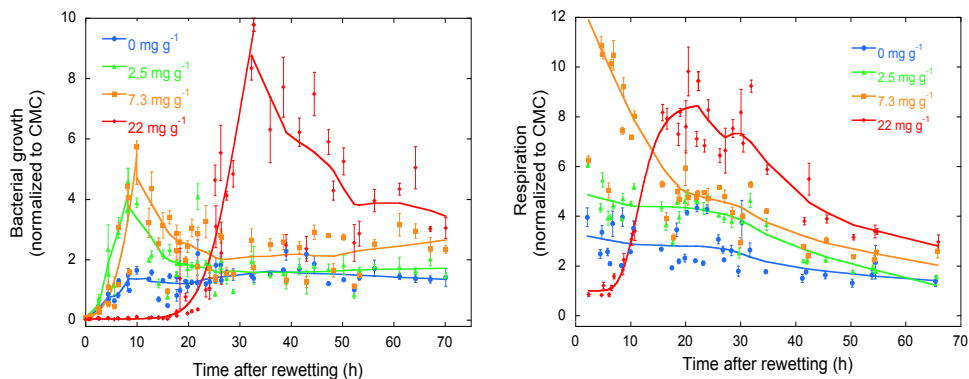
Fig. 14
Bacterial growth (left panel) and respiration (right panel) after rewetting of dried soils receiving 0, 2.5, 7.3 and 22 mg NaCl g^{-1}. Bacterial growth and respiration rates have been normalized to the rates measured in the continuously moist control soilat each salinity level. Data points show the mean (n=3) and the standard error (Paper VI).

Synthesis

To understand the microbial role in controlling biogeochemical cycling, we need to understand how environmental factors impact microbial functioning and community structure. Important environmental factors include factors that are related to soil chemistry. Salinity is such a chemical environmental factor that is easily modified under laboratory conditions and in the field. Exposure to increased salt concentrations is reversible, by leaching out salt or inoculating communities into new soil. In addition, natural salinity gradients can cover vast spans of salinity, from non-saline to hypersaline, in a small geographical space, keeping the influence of spatial factors on microbial communities to a minimum. This makes salinity a good model factor to test how abiotic factors regulate biogeochemical processes in soil through their influence on the microbial community.

This thesis demonstrates that salinity is of major importance in controlling microbial process rates in soils, by reducing both microbial growth and mineralization of C. Salinity was also shown to modify the microbial community composition, by changing the fungal/bacterial ratio, as well as leading to large shifts in the abundance of bacterial taxa. This effect was of comparable magnitude to that of pH, often considered one of the most important environmental factors in soil (Lauber et al., 2009). Considering the extent of salt-affected areas globally,

the effect of salinity on C cycling and storage as well as on other biogeochemical processes in soil should be taken into account. Changes in the C content of soils in response to salinization could have implications for C sequestration in soils. Current models of soils organic carbon stocks do not account for the impacts of soil salinization, and could thus wrongly estimate the future development of soil C stocks (Setia et al., 2013). Including the effects of salinity on both plants and soil microorganisms could improve soil C stock projections, particularly in arid and semi-arid areas.

This thesis included studies conducted across three different time scales: (i) short-term assessments with salt exposure on the time scale of hours (Paper II), (ii) microcosm studies with a duration of 1-2 months (Paper III, VI) and (iii) natural salinity gradients with salt exposure over decades to centuries (Paper IV, V). In short-term assessments, processes were strongly affected by salt additions. For instance, one hour after addition of 20 mg NaCl g^{-1} soil, respiration was only 20% of the rate measured in a control without salt (Paper II). When the community was given time to adapt for about a month, respiration in the treatment receiving 20 mg g^{-1} NaCl was about 40% of the rate measured in a non-saline soil (Paper III). This was the result of a shift towards higher community salt tolerance that occurred in less than a week. Tolerances that were reached in non-saline soil within a month were of similar magnitude as tolerances found in soils from a natural salinity gradient that had been exposed to these salinities for long time periods (Paper IV). An interesting question is to compare the potential of soils with different legacies of salt exposure to adapt to higher salinities. It is possible that communities originating from saline soil have a higher functional potential to adapt to increasing salt concentrations than communities from non-saline soil. Consequently these better-adapted communities could be able to maintain higher performance at increasing salinity. Conversely, by selecting for slower growing microorganisms and decreasing the diversity of the community, salinity could have a lasting impact on microbial functioning even after the salt disturbance has been removed.

Acquiring a predictive understanding of the response of the microbial community composition to environmental change is of central importance in microbial ecology. The performance of microorganisms along gradients of salinity depends largely on their salt tolerance, making salt tolerance an important functional trait. Since trait values for individual microbial species are largely unknown, we quantified the distribution of the functional traits aggregated at the community level as dose-response relationships of whole communities (Paper IV). By quantifying the effect of salinity and pH on filtering the distribution of the functional traits salt tolerance and pH tolerance in soil microbial communities, we could detect strong impacts of both factors on driving soil microbial community

composition along the environmental gradients. This allowed for the identification of potential biomarker taxa that could be used to infer ecosystem function. If this information were to be collected in databases, multiple reported observations could result in an accumulation of data that would ultimately serve to predict functional properties of a community from sequencing data.

The type of disturbance that salinity poses to microbial communities in the environment is a press disturbance, in that its duration is sustained beyond the initial disturbance. This is in contrast to a short-term pulse disturbance such as drought, in which environmental conditions after a while return to their initial state. In the drying-rewetting experiment (Paper VI) we combined the press disturbance of salinity with the pulse disturbance of drying, followed by rewetting of dried soil to the initial water content. Salinity increased the severity of the impact that drying had on the microbial community, thereby modifying the response of the community to drying-rewetting. Other press disturbances, such as heavy metal pollution of soil, could similarly exacerbate the effect of pulse disturbances. Soil ecosystems that suffer from chronic disturbances could consequently be less resistant to pulse disturbances.

In response to drying-rewetting of saline soil we saw a delay in the respiration pulse after rewetting, which coincided with a lag in the growth rate (**Fig. 14**). This type of a respiration response had not been observed previously, but respiration responses to drying-rewetting in saline soils had not been measured at high time resolution before. It is possible that as salts accumulated in the remaining soil water during drying, enzymes in soil got denatured ("salted out") and upon rewetting, inhibited enzyme activity resulted in initially low respiration, until the enzyme pool in the soil got replenished. High time resolution measurements of enzyme activities in soils of different salinities during drying-rewetting could elucidate the role of enzymes in the respiration pulse in rewetted soil.

Irrespective of the time scales used for studying the effect of salinity, fungi were found to be more resistant to salinity than bacteria. In the short-term assessments fungi were slightly less inhibited by added salt than bacteria (Paper II). In the medium-term microcosm experiments fungi could recover better than bacteria following high salt additions (Paper III). Also along natural salinity gradients where communities had been exposed to long-term salinization, fungal growth was less affected by salinity than bacterial growth (Paper IV). All of this demonstrates that fungi are better able to withstand high salinity than bacteria. This goes against the conventional wisdom that fungi are disfavored by salinity, which is primarily based on their low abundance in many highly saline habitats, both terrestrial and aquatic (Gunde-Cimerman et al., 2009). However, our finding that fungi in soil have higher resistance to salinity suggests that this low

abundance is unlikely to be a result of salinity directly, and instead could be caused by other factors correlating with salinity. For instance, highly saline habitats are often low in complex organic material, of which fungi are the primary decomposers. In addition, many hypersaline habitats have a high pH, which favours bacteria (Rousk et al., 2010). Our results raise the possibility that fungal-dominated systems have a higher resistance to salt exposure. If that is the case, agricultural management that is set to promote fungal dominance of soils, e.g. by addition of high C/N plant litter (Rousk and Bååth, 2007a) or reduction of tillage, could lead to development of more salt-resistant microbial communities.

Outlook

The impact of soil salinity on microbial process rates is of major importance when considering future soil fertility of salt-affected areas, since microorganisms are essential for nutrient cycling and nutrient retention in soil. A lot of research efforts are focused on improving salt tolerance in crop plants through breeding and genetic engineering of more salt tolerant crop varieties (Chinnusamy et al., 2005). Salt-tolerant crops have been identified as a promising avenue forward to increase yields and improve food security in salt-affected areas (Ashraf et al., 2008). However, for the cultivation of salt tolerant varieties to be successful, the functions carried out by soil microorganisms that are vital for the maintenance of soil fertility need to be upheld in salt-affected soils. The findings in this thesis show that the induction of community salt tolerance in the microbial community in response to salt can quickly alleviate some of the loss of functioning of the microbial community that is expected in response to salt exposure (Paper III, Paper IV). Organic matter additions, which in the form of straw or manure are a frequently used amelioration strategy for soil salinity, could also improve microbial functioning in saline soils (Paper V). However, even after addition of organic substrate microbial process rates in saline soils were still reduced compared to saline soils, and diversity of microbial communities declined in response to salinity (Paper IV, Paper V). Therefore, to develop sustainable agricultural management practices in salt-affected soils, the potential of the soil microbial community to maintain functioning has to be considered.

References

Anderson, J.P.E., Domsch, K.H., 1978. Physiological Method for Quantitative Measurement of Microbial Biomass in Soils. Soil Biology & Biochemistry 10, 215-221.

Ashraf, M., Athar, H.R., Harris, P.J.C., Kwon, T.R., 2008. Some prospective strategies for improving crop salt tolerance. Advances in Agronomy, Vol 97 97, 45-110.

Bååth, E., 2001. Estimation of fungal growth rates in soil using C-14-acetate incorporation into ergosterol. Soil Biology & Biochemistry 33, 2011-2018.

Bååth, E., Pettersson, M., Söderberg, K.H., 2001. Adaptation of a rapid and economical microcentrifugation method to measure thymidine and leucine incorporation by soil bacteria. Soil Biology & Biochemistry 33, 1571-1574.

Berga, M., Zha, Y.H., Szekely, A.J., Langenheder, S., 2017. Functional and Compositional Stability of Bacterial Metacommunities in Response to Salinity Changes. Frontiers in Microbiology 8.

Bernstein, L., 1975. Effects of Salinity and Sodicity on Plant-Growth. Annual Review of Phytopathology 13, 295-312.

Birch, H.F., 1958. The effect of soil drying on humus decomposition and nitrogen availability. Plant and Soil 10, 9-31.

Blagodatskaya, E., Kuzyakov, Y., 2013. Active microorganisms in soil: Critical review of estimation criteria and approaches. Soil Biology & Biochemistry 67, 192-211.

Blanck, H., Wängberg, S.A., 1988. Induced community tolerance in marine periphyton established under arsenate stress. Canadian Journal of Fisheries and Aquatic Sciences 45, 1816-1819.

Chinnusamy, V., Jagendorf, A., Zhu, J.K., 2005. Understanding and improving salt tolerance in plants. Crop Science 45, 437-448.

Chowdhury, N., Marschner, P., Burns, R., 2011. Response of microbial activity and community structure to decreasing soil osmotic and matric potential. Plant and Soil 344, 241-254.

Ciais, P., Sabine, C., Bala, G., Bopp, L., Brovkin, V., Canadell, J., Chhabra, A., DeFries, R., Galloway, J., Heimann, M., Jones, C., Le Quéré, C., Myneni, R.B., Piao, S., Thornton, P., 2013. Carbon and Other Biogeochemical Cycles, In: Stocker, T.F., Qin, D., Plattner, G.-K., Tignor, M., Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex, V., Midgley, P.M. (Eds.), Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp. 465–570.

Daliakopoulos, I.N., Tsanis, I.K., Koutroulis, A., Kourgialas, N.N., Varouchakis, A.E., Karatzas, G.P., Ritsema, C.J., 2016. The threat of soil salinity: A European scale review. Science of The Total Environment 573, 727-739.

de Boer, W., Folman, L.B., Summerbell, R.C., Boddy, L., 2005. Living in a fungal world: impact of fungi on soil bacterial niche development. FEMS microbiology reviews 29, 795-811.

FAO, 2011. The state of the world's land and water resources for food and agriculture : managing systems at risk, 1st ed. Earthscan, Milton Park, Abingdon ; New York, NY.

Fierer, N., Colman, B.P., Schimel, J.P., Jackson, R.B., 2006. Predicting the temperature dependence of microbial respiration in soil: A continental-scale analysis. Global Biogeochemical Cycles 20.

Fischer, G., Nachtergaele, F., Prieler, S., van Velthuizen, H.T., Vereist, L., Wiberg, D., 2008. Global Agro-ecological Zones Assessment for Agriculture (GAEZ 2008), IIASA, Laxenburg, Austria and FAO, Rome, Italy.

Frostegård, A., Bååth, E., 1996. The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. Biology and Fertility of Soils 22, 59-65.

Grant, W.D., 2004. Life at low water activity. Philosophical Transactions of the Royal Society of London Series B-Biological Sciences 359, 1249-1266.

Green, J.L., Bohannan, B.J.M., Whitaker, R.J., 2008. Microbial biogeography: From taxonomy to traits. Science 320, 1039-1043.

Guggenberger, G., Frey, S.D., Six, J., Paustian, K., Elliott, E.T., 1999. Bacterial and fungal cell-wall residues in conventional and no-tillage agroecosystems. Soil Science Society of America Journal 63, 1188-1198.

Gunde-Cimerman, N., Ramos, J., Plemenitas, A., 2009. Halotolerant and halophilic fungi. Mycological Research 113, 1231-1241.

Gunde-Cimerman, N., Zalar, P., de Hoog, S., Plemenitas, A., 2000. Hypersaline waters in salterns - natural ecological niches for halophilic black yeasts. Fems Microbiology Ecology 32, 235-240.

Hagerty, S.B., van Groenigen, K.J., Allison, S.D., Hungate, B.A., Schwartz, E., Koch, G.W., Kolka, R.K., Dijkstra, P., 2014. Accelerated microbial turnover but constant growth efficiency with warming in soil. Nature Climate Change 4, 903-906.

Hasegawa, P.M., Bressan, R.A., Zhu, J.K., Bohnert, H.J., 2000. Plant cellular and molecular responses to high salinity. Annual Review of Plant Physiology and Plant Molecular Biology 51, 463-499.

Hatton, T.J., Ruprecht, J., George, R.J., 2003. Preclearing hydrology of the Western Australia wheatbelt: Target for the future? Plant and Soil 257, 341-356.

Jobbagy, E.G., Jackson, R.B., 2004. Groundwater use and salinization with grassland afforestation. Global Change Biology 10, 1299-1312.

Joergensen, R.G., 1996. The fumigation-extraction method to estimate soil microbial biomass: Calibration of the k(EC) value. Soil Biology & Biochemistry 28, 25-31.

Kakumanu, M.L., Williams, M.A., 2014. Osmolyte dynamics and microbial communities vary in response to osmotic more than matric water deficit gradients in two soils. Soil Biology and Biochemistry 79, 14-24.

Kamble, P.N., Gaikwad, V.B., Kuchekar, S.R., Bååth, E., 2014. Microbial growth, biomass, community structure and nutrient limitation in high pH and salinity soils from Pravaranagar (India). European Journal of Soil Biology 65, 87-95.

Kirschbaum, M.U.F., 1995. The Temperature-Dependence of Soil Organic-Matter Decomposition, and the Effect of Global Warming on Soil Organic-C Storage. Soil Biology & Biochemistry 27, 753-760.

Lal, R., 2004. Soil carbon sequestration impacts on global climate change and food security. Science 304, 1623-1627.

Lauber, C.L., Hamady, M., Knight, R., Fierer, N., 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Applied and Environmental Microbiology 75, 5111-5120.

Liang, C., Schimel, J.P., Jastrow, J.D., 2017. The importance of anabolism in microbial control over soil carbon storage. Nature Microbiology 2.

Lloyd, J., Taylor, J.A., 1994. On the Temperature-Dependence of Soil Respiration. Functional Ecology 8, 315-323.

Manzoni, S., Taylor, P., Richter, A., Porporato, A., Agren, G.I., 2012. Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. New Phytologist 196, 79-91.

Mavi, M.S., Marschner, P., 2012. Drying and wetting in saline and saline-sodic soils-effects on microbial activity, biomass and dissolved organic carbon. Plant and Soil 355, 51-62.

McGuire, K.L., Treseder, K.K., 2010. Microbial communities and their relevance for ecosystem models: Decomposition as a case study. Soil Biology & Biochemistry 42, 529-535.

Meisner, A., Bååth, E., Rousk, J., 2013. Microbial growth responses upon rewetting soil dried for four days or one year. Soil Biology & Biochemistry 66, 188-192.

Meisner, A., Rousk, J., Bååth, E., 2015. Prolonged drought changes the bacterial growth response to rewetting. Soil Biology & Biochemistry 88, 314-322.

Montgomery, H.J., Monreal, C.M., Young, J.C., Seifert, K.A., 2000. Determination of soil fungal biomass from soil ergosterol analyses. Soil Biology & Biochemistry 32, 1207-1217.

Nunan, N., Wu, K.J., Young, I.M., Crawford, J.W., Ritz, K., 2003. Spatial distribution of bacterial communities and their relationships with the micro-architecture of soil. Fems Microbiology Ecology 44, 203-215.

Oren, A., 1999. Bioenergetic aspects of halophilism. Microbiology and Molecular Biology Reviews 63, 334-+.

Oren, A., 2011. Thermodynamic limits to microbial life at high salt concentrations. Environmental Microbiology 13, 1908-1923.

Pankhurst, C.E., Yu, S., Hawke, B.G., Harch, B.D., 2001. Capacity of fatty acid profiles and substrate utilization patterns to describe differences in soil microbial communities associated with increased salinity or alkalinity at three locations in South Australia. Biology and Fertility of Soils 33, 204-217.

Perlin, J., 1989. A forest journey : the role of wood in the development of civilization, 1st ed. W.W. Norton, New York.

Pitman, M.G., Läuchli, A., 2002. Global impact of salinity and agricultural ecosystems, In: Läuchli, A., Luettge, U. (Eds.), Salinity: Environment - Plants - Molecules. Kluwer Academic Publishers, Dordrecht, Germany.

Qadir, M., Quillerou, E., Nangia, V., Murtaza, G., Singh, M., Thomas, R.J., Drechsel, P., Noble, A.D., 2014. Economics of salt-induced land degradation and restoration. Natural Resources Forum 38, 282-295.

Rajapaksha, R.M.C.P., Tobor-Kaplon, M.A., Baath, E., 2004. Metal toxicity affects fungal and bacterial activities in soil differently. Applied and Environmental Microbiology 70, 2966-2973.

Rath, K.M., Rousk, J., 2015. Salt effects on the soil microbial decomposer community and their role in organic carbon cycling: A review. Soil Biology & Biochemistry 81, 108-123.

Reed, H.E., Martiny, J.B.H., 2007. Testing the functional significance of microbial composition in natural communities. Fems Microbiology Ecology 62, 161-170.

Rengasamy, P., 2006. World salinization with emphasis on Australia. Journal of Experimental Botany 57, 1017-1023.

Richards, L.A., 1954. Diagnosis and improvement of saline and alkali soils. Soil Science 78, 154.

Romani, A.M., Fischer, H., Mille-Lindblom, C., Tranvik, L.J., 2006. Interactions of bacteria and fungi on decomposing litter: Differential extracellular enzyme activities. Ecology 87, 2559-2569.

Rousk, J., 2016. Biomass or growth? How to measure soil food webs to understand structure and function. Soil Biology & Biochemistry 102, 45-47.

Rousk, J., Bååth, E., 2007a. Fungal and bacterial growth in soil with plant materials of different C/N ratios. Fems Microbiology Ecology 62, 258-267.

Rousk, J., Bååth, E., 2007b. Fungal biomass production and turnover in soil estimated using the acetate-in-ergosterol technique. Soil Biology & Biochemistry 39, 2173-2177.

Rousk, J., Bååth, E., Brookes, P.C., Lauber, C.L., Lozupone, C., Caporaso, J.G., Knight, R., Fierer, N., 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil. Isme Journal 4, 1340-1351.

Schimel, J.P., Gulledge, J.M., Clein-Curley, J.S., Lindstrom, J.E., Braddock, J.F., 1999. Moisture effects on microbial activity and community structure in decomposing birch litter in the Alaskan taiga. Soil Biology & Biochemistry 31, 831-838.

Schlesinger, W.H., Andrews, J.A., 2000. Soil respiration and the global carbon cycle. Biogeochemistry 48, 7-20.

Schmidt, M.W.I., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I.A., Kleber, M., Kogel-Knabner, I., Lehmann, J., Manning, D.A.C., Nannipieri, P., Rasse, D.P., Weiner, S., Trumbore, S.E., 2011. Persistence of soil organic matter as an ecosystem property. Nature 478, 49-56.

Setia, R., Gottschalk, P., Smith, P., Marschner, P., Baldock, J., Setia, D., Smith, J., 2013. Soil salinity decreases global soil organic carbon stocks. Science of The Total Environment 465, 267-272.

Setia, R., Marschner, P., Baldock, J., Chittleborough, D., Smith, P., Smith, J., 2011. Salinity effects on carbon mineralization in soils of varying texture. Soil Biology & Biochemistry 43, 1908-1916.

Six, J., Frey, S.D., Thiet, R.K., Batten, K.M., 2006. Bacterial and fungal contributions to carbon sequestration in agroecosystems. Soil Science Society of America Journal 70, 555-569.

Sollins, P., Kramer, M.G., Swanston, C., Lajtha, K., Filley, T., Aufdenkampe, A.K., Wagai, R., Bowden, R.D., 2009. Sequential density fractionation across soils of contrasting mineralogy: evidence for both microbial- and mineral-controlled soil organic matter stabilization. Biogeochemistry 96, 209-231.

Spohn, M., Klaus, K., Wanek, W., Richter, A., 2016. Microbial carbon use efficiency and biomass turnover times depending on soil depth - Implications for carbon cycling. Soil Biology & Biochemistry 96, 74-81.

Strickland, M.S., Lauber, C., Fierer, N., Bradford, M.A., 2009. Testing the functional significance of microbial community composition. Ecology 90, 441-451.

Strickland, M.S., Rousk, J., 2010. Considering fungal:bacterial dominance in soils - Methods, controls, and ecosystem implications. Soil Biology & Biochemistry 42, 1385-1395.

Szabolcs, I., 1989. Salt-Affected Soils. C R C Press LLC.

Tavakkoli, E., Rengasamy, P., McDonald, G.K., 2010. High concentrations of Na+ and Cl- ions in soil solution have simultaneous detrimental effects on growth of faba bean under salinity stress. Journal of Experimental Botany 61, 4449-4459.

Tejada, M., Garcia, C., Gonzalez, J.L., Hernandez, M.T., 2006. Use of organic amendment as a strategy for saline soil remediation: Influence on the physical, chemical and biological properties of soil. Soil Biology & Biochemistry 38, 1413-1421.

Tester, M., Davenport, R., 2003. Na+ tolerance and Na+ transport in higher plants. Annals of Botany 91, 503-527.

Van de Noort, R., 2013. Climate change archaeology : building resilience from research in the world's coastal wetlands, First edition. ed. Oxford University Press, Oxford.

Wallenstein, M.D., Hall, E.K., 2012. A trait-based framework for predicting when and where microbial adaptation to climate change will affect ecosystem functioning. Biogeochemistry 109, 35-47.

Wardle, D.A., Ghani, A., 1995. A critique of the microbial metabolic quotient (qCO(2)) as a bioindicator of disturbance and ecosystem development. Soil Biology & Biochemistry 27, 1601-1610.

Webb, C.T., Hoeting, J.A., Ames, G.M., Pyne, M.I., Poff, N.L., 2010. A structured and dynamic framework to advance traits-based theory and prediction in ecology. Ecology Letters 13, 267-283.

Wichern, J., Wichern, F., Joergensen, R.G., 2006. Impact of salinity on soil microbial communities and the decomposition of maize in acidic soils. Geoderma 137, 100-108.

Wicke, B., Smeets, E., Dornburg, V., Vashev, B., Gaiser, T., Turkenburg, W., Faaij, A., 2011. The global technical and economic potential of bioenergy from salt-affected soils. Energy & Environmental Science 4, 2669-2681.

Wong, V.N.L., Greene, R.S.B., Dalal, R.C., Murphy, B.W., 2010. Soil carbon dynamics in saline and sodic soils: a review. Soil Use and Management 26, 2-11.

Wood, J.M., 2011a. Bacterial Osmoregulation: A Paradigm for the Study of Cellular Homeostasis. Annual Review of Microbiology, Vol 65 65, 215-238.

Wood, J.M., 2011b. Osmotic Stress, In: Storz, G., Hengge, R. (Eds.), Bacterial Stress Responses, 2nd edition ed. ASM Press, Washington, DC.

CrossMark

Review paper

Salt effects on the soil microbial decomposer community and their role in organic carbon cycling: A review

Kristin M. Rath [a, b], Johannes Rousk [a, *]

[a] Section of Microbial Ecology, Department of Biology, Lund University, 22362 Lund, Sweden
[b] Centre for Environmental and Climate Research (CEC), Lund University, Lund, Sweden

ARTICLE INFO

ABSTRACT

Salinization of soil is recognised as one of the most pressing environmental challenges to resolve for the next century. We here conduct a synoptic review of the available research on how salt affects decomposer microbial communities and carbon (C) cycling in soil. After summarizing known physiological responses of microorganisms to salinity, we provide a brief overview and qualification of a selection of widely applied methods to assess microorganisms in soil to date. The dominant approaches to characterise microbial responses to salt exposure have so far been microbial biomass and respiration measurements. We compile datasets from a selection of studies and find that (1) microbial biomass-carbon (C) per C held in soil organic matter shows no consistent pattern with long-term (field gradients) or short-term (laboratory additions) soil salinity level, and (2) respiration per soil organic C is substantially inhibited by higher salt concentrations in soil, and consistently so for both short-term and long-term salinity levels. Patterns that emerge from extra-cellular enzyme assessments are more difficult to generalize, and appear to vary with the enzyme studied, and its context. Growth based assessments of microbial responses to salinization are largely lacking. Relating the established responses of microbial respiration to that of growth could provide an estimate for how the microbial C-use efficiency would be affected by salt exposure. This would be a valuable predictor for changes in soil C sequestration. A few studies have investigated the connection between microbial tolerance to salt and the soil salinity levels, but so far results have not been conclusive. We predict that more systematic inquiries including comprehensive ranges of soil salinities will substantiate a connection between soil salinity and microbial tolerance to salt. This would confirm that salinity has a direct effect on the composition of microbial communities. While salt has been identified as one of the most powerful environmental factors to structure microbial communities in aquatic environments, no up-to-date sequence based assessments currently exist from soil. Filling this gap should be a research priority. Moreover, linking sequencing based assessments of microbial communities to their tolerance to salt would have the potential to yield biomarker sets of microbial sequences. This could provide predictive power for, e.g., the sensitivity of agricultural soils to salt exposure, and, as such, a useful tool for soil resource management. We conclude that salt exposure has a powerful influence on soil microbial communities and processes. In addition to being one of the most pressing agricultural problems to solve, this influence could also be used as an experimental probe to better understand how microorganisms control the biogeochemistry in soil.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

The United Nations Rio+20 summit initiated the process to update the Millenium Development Goals, and committed the member nations to create new Sustainable Development Goals (SDG) for the new century. Recently, a first attempt strove to identify and distil out a tentative list (Griggs et al., 2013). This work stressed that most central for achieving a sustainable planet is the stable functioning of the Earth systems – including biodiversity and biogeochemical cycles. A sustainable planet must build on this foundation. Out of six identified targets for 2030 by the pioneering authors, three – sustainable food security, sustainable water security, and sustaining biodiversity and ecosystem services – are directly dependent on a mechanistic understanding for how the microbial regulation of soil biogeochemistry is affected by salinization.

* Corresponding author. Tel.: +46 46 222 37 45.
  E-mail address: johannes.rousk@biol.lu.se (J. Rousk).

Soil salinization is a term used for the accumulation of salt in soils at a level that negatively impacts agricultural productivity, environmental health and economic welfare (Rengasamy, 2006b). Generally, a soil is described as saline if the electrical conductivity measured in a saturated soil paste (EC_e) is higher than 4 dS m^{-1} (US Laboratory Staff, 1954). The Food and Agriculture Organisation of the United Nations (FAO) estimates that globally over 830 M ha of arable land are affected by salinization (Szabolcs, 1989; Martinez-Beltran and Manzur, 2005), corresponding to about 10% of the globe's arable land (Szabolcs, 1989). Salinization affects up to 3 M ha land in Europe, the 17 western states of the USA, >5% of the land in Africa, about a fifth of the arable land of West Asia, and 30% of the Australian land area (Chhabra, 1996; Rengasamy, 2006b; UNEP, 2007; Ladeiro, 2012), making it a world-wide environmental challenge. Of the global threats that collectively compromise about 10 ha arable land per minute (Griggs et al., 2013), salinization contributes about 30% (Buringh, 1978).

In the context of this review on the effects of salt, we refer to salts as ionic compounds composed of an equal number of anions and cations. Ionic bonds between the oppositely charged ions form through electrostatic attraction. Most salts are easily soluble in water, leading to the presence of ions in solution. In soils, the pore water contains a variety of dissolved ions such as Na^+, Ca^{2+}, NH_4^+, Cl^- or SO_4^{2-}. As soil water content decreases, dissolved ions become more concentrated. Salt accumulation in the surface soil is often found in agricultural areas in arid and semi-arid regions, where it is caused by irrigation with brackish or saline water in poorly drained soils (Allison, 1964). In areas with a shallow groundwater body evaporating ground water can also lead to higher salt concentration in the soil surface layer (Rengasamy, 2006b). In addition, soil salinization can be the result of changes in vegetation cover that alter ecosystem water balances. For instance, in Australia extensive areas are undergoing dryland salinization as a consequence of the replacement of native, deep-rooted perennial vegetation with shallow-rooted agricultural plants. This led to lower evapotranspiration of rainfall and waterlogging of areas with saline groundwater (Clarke et al., 2002). A vegetation change in the opposite direction, from grassland to forest, can also lead to soil salinization, when evapotranspiration exceeds groundwater recharge (Jobbagy and Jackson, 2004; Jackson et al., 2005). Salt water intrusion from marine environments is also an important cause for soil salinization (Chandrajith et al., 2014), which has resulted in the salinization of 53% of coastal regions in e.g. Bangladesh (Haque, 2006).

Salinization leads to physical changes in soil. High salt concentrations result in flocculation or dispersion of soil particles, which influences soil organic matter (SOM) solubility (Shainberg and Letey, 1984; Wong et al., 2009, 2010). In addition, the type of salt predominantly present in the soil also plays a role in determining SOM solubility. Multivalent cations in the soil solution, such as Ca^{2+}, can link together negatively charged clay particles and organic compounds (Oades, 1984). Thereby the presence of multivalent cations can increase the sorption of organic matter to soil particles (Mikutta et al., 2007; Mavi et al., 2012) and thus reduce the amount of organic matter available for decomposition (Oades, 1988; Six et al., 2000). Monovalent cations such as Na^+ form much weaker bonds. Soils with a high concentration of Na^+ are called sodic soils. Soils are normally considered to be sodic if they have a sodium absorption ratio (SAR) > 13. If a sodic soil also has an electrical conductivity >4 dS m^{-1} it is classified as saline-sodic (US Salinity Laboratory Staff, 1954). In sodic soils the sorption of organic compounds to the soil matrix is reduced (Setia et al., 2013). If a higher percentage of exchange sites are occupied by monovalent cations, cross-linking between organic molecules and mineral surfaces is decreased. Soils become more liable to erosion and leaching, and organic matter is less protected from decomposition (Sumner and Naida, 1998; Wong et al., 2010). Dispersion of soil particles can also affect oxygen availability with consequences for microbial activity (Bronick and Lal, 2005).

Soil salinization naturally has direct impacts on plants, and has subsequently been a research priority for crops for decades (Ayers and Westcot, 1976; Chhabra, 1996; Katerji et al., 2003; Arshad, 2008; Stevens and Partington, 2013). For instance, salt exposure is known to reduce crop yield under greenhouse and field conditions in e.g. barley (Pal et al., 1984; Richards et al., 1987), wheat (Richards, 1983; Bajwa et al., 1986), cotton (Meloni et al., 2001; Soomro et al., 2001), sugar cane (Choudhary et al., 2004), rice (Bajwa et al., 1986), maize (Bajwa et al., 1986) and sugar beet (Ghoulam et al., 2002). Crops and cultivars differ in their tolerance to salinity, and this is also modulated by environmental and soil factors. Furthermore, indirect consequences of salinization are ion imbalance and nutrient deficiency (Marschner, 1995), further aggravating the negative effects on plant productivity. Although crop resistance to salt exposure is a promising development (e.g. Bennett et al., 2013), overall plant productivity will be impeded by salinization. However, less is known about the effects of salinity on soil microorganisms. This review will therefore focus on responses of the microbial decomposer community and soil C cycling to salinity.

Soil is the habitat for a huge concentration of microorganisms. According to estimates, 1 g of soil contains up to ten billion bacterial cells (Torsvik and Ovreas, 2002; Horner-Devine et al., 2004) and kilometres of fungal hyphae (Bååth and Söderström, 1979; De Boer et al., 2005). Microorganisms are the principal drivers of all nutrient cycles, and especially for the decomposition of SOM, thereby regenerating plant nutrients. Consequently, any effects by salt on microbial processes will have large implications for SOM dynamics, ecosystem biogeochemical cycling, and plant nutrition (Marschner, 1995; Raich and Potter, 1995; Schlesinger, 1997; Rustad et al., 2000; Setia et al., 2010; Setia et al., 2012). SOM decomposition is influenced by a range of abiotic and biotic factors, such as temperature and moisture (Waksman and Gerretsen, 1931; Davidson and Janssens, 2006), as well as pH (Blagodatskaya and Anderson, 1998; Rousk et al., 2011a), redox conditions (Schmidt et al., 2011) and the community composition of microbes, plants and fauna (Wardle et al., 2004). Salinity is an environmental factor that is receiving increasing attention, but our understanding of the effect of soil salt concentrations on the structure and functioning of the soil microbial community is still fragmented and incomplete.

With this review we intend to provide a synoptic review of the available literature to date on how salt exposure influences decomposer microorganisms and decomposer microbial processes relating to the C cycle in soil. While comprehensive reviews on the effects of salinization on the soil N cycle, along with literature reviews on how salinization effects can be mitigated through land management are not available, either of these subjects deserve separate treatment and will and should encompass extensive research compilations. Furthermore, it should be noted that the ecosystem consequences of salinization will be a balanced outcome of the effects on both the plant community and the below-ground soil microbial decomposer community. This review will not specifically focus on the effects on the plant community, and for interested readers we refer to an already existent body of work in e.g. Zhu (2001) and Parida and Das (2005).

Our endeavour to review salt effects on the soil microbial decomposer community and C cycling necessitates brief summaries of how salinity can affect the microbial physiology, along with an overview and qualification of current methods used to assess microorganisms in salt-exposed soil. We will review what insights systematic application of these methods has revealed about the

influence of salt on soil decomposer microorganisms. Further, we will query this overview for general insights about microbial responses to salt, by combining available literature results into consensus analyses. We intend to bridge our survey into subject areas related to soil microbial ecology, including aquatic microbial ecology and ecotoxicology. Finally, we will identify knowledge gaps and research priorities, thereby formulating useful research questions that should be prioritized. We hope this can contribute to generating progress in the understanding of how decomposer microorganisms respond to the salinization of soil.

2. Review

2.1. Physiological effects of salinity on microorganisms

Increasing salt concentrations in the environment elevate the osmolarity outside the microbial cell. As external salt concentrations rise, cells loose water. In order to maintain cell turgor and prevent dehydration microorganisms have been shown to accumulate and produce osmolytes in their cytoplasm (Empadinhas and da Costa, 2008). Two different strategies to achieve homeostasis with the surrounding environment exist: First, there are a number of halophilic prokaryotes that take up ions, predominantly potassium ions. This, however, requires adaptations of intracellular enzymes to high ion concentrations in the cytoplasm, since proteins should be able to maintain their proper conformation and activity at high cytoplasmatic salt concentrations (Oren, 2008). Not all ions are accumulated at equal rates. Less toxic ions such as K^+ are favoured over Na^+ ions through selective ion pumping (Ventosa et al., 1998). As an example, cytoplasmatic Na^+ concentrations in halophilic bacteria are frequently lower than in the surrounding medium. This means that Na^+ has to be exported from the cell against its concentration gradient, which is achieved by Na^+/H^+ antiport activity or respiration-driven Na^+ pumps. At the same time K^+ must be accumulated inside the cell against a concentration gradient. The second, more widespread strategy is the accumulation of low molecular weight organic compounds such as amino acids and carbohydrates within the cell (Kempf and Bremer, 1998; Oren, 2008). Both osmo-adaptation strategies are energetically expensive (Oren, 1999). The synthesis of organic osmolytes requires energy in the form of ATP. Oren (1999) calculated that heterotrophic microorganisms need to use between 23 and 79 ATP molecules to produce one molecule of an osmotic solute. Extrusion of Na^+ and uptake of K^+ also consumes ATP equivalents (Oren, 1999). Moreover, salt concentrations in the soil pore water are rarely constant over time but subject to fluctuations, increasing the need for regulation. In response to decreasing osmotic pressure microorganisms must also be able to adjust intracellular osmolarity by expulsion of osmolytes.

In many arid and semiarid areas affected by salinization, high salt concentrations are often combined with low availability of water. Both osmotic and matric potential contribute to the total soil water potential (Kakumanu and Williams, 2014). As matric potential decreases during drying of soil, water is held more tightly to soil aggregates and its availability for soil organisms decreases. With declining water potential microbes are facing dehydration. In addition, dissolved salts in the pore water also become more concentrated. Drought conditions therefore intensify the effects of salinity on microbes, and it can be difficult to clearly separate the effects of declining matric potential and osmotic potential (Chowdhury et al., 2011a). Microbes are thought to react to drought in a similar way as to high salt concentrations, by accumulating osmolytes inside the cell (Mikha et al., 2005; Schimel et al., 2007). Studies on soil, however, have for the most part failed to detect an increase of probable osmolytes in response to soil drying (Williams

and Xia, 2009; Göransson et al., 2013; Kakumanu et al., 2013). However, Warren (2014) recently observed an increase of known microbial osmolytes such as ectoine, hydroxyectoine, betaine and arabitol in extracts from soils experimentally subjected to drought. Differences in used experimental conditions, such as incubation time, could have contributed to observed differences between studies. Williams and Xia (2009) and Kakumanu et al. (2013) dried soils for only 3–4 days, which might not have been enough time for microorganisms to accumulate osmolytes, whereas Warren (2014) let soils dry for several months. In bacterial cultures, however, osmolyte production can be observed within hours after increasing salinity levels (Bursy et al., 2008). While 4 days was found to be enough time for cultured bacteria to increase intracellular amino acid concentrations in response to osmotic pressure (Killham and Firestone, 1984), diffusion limitation in drying soils might restrict access to resources necessary for osmolyte synthesis. Recent reports suggest that osmolyte responses to salt are more pronounced than to corresponding changes in water potential, possibly due to restricted resource transport through the soil matrix in the latter case (Kakumanu and Williams, 2014).

Energetically expensive adaptation mechanisms divert resources from growth to survival mechanisms (Schimel et al., 2007). If organisms fail to cope with environmental conditions they die or become inactive, thus altering the composition of the community (Placella et al., 2012). Both physiological adaptations and changes in microbial community composition towards a more tolerant community have an impact on microbial functioning and can change energy and nutrient fluxes in the environment. For instance, communities that had been exposed to a period of drought prior to the measurements (performed at standardised moisture) have been found to show lower organic matter (OM) mineralization rates (Schimel et al., 1999; Fierer and Schimel, 2002). Additionally, field drought treatments have been found to reduce mineralisation during a rewetting event more than microbial growth (Göransson et al., 2013). This could suggest that microbial growth efficiencies could increase following a shift toward a community better adapted to use C efficiently — allocating more resources to growth — under the changed environmental conditions. However, the outcome is of course also susceptible to how the plant C allocation below-ground responds, which will influence the microbial community (Wichern et al., 2004).

2.2. Methodological considerations when assessing soil microbial communities

The microbial decomposer community can be divided into two large groups: bacteria and fungi. Some fungi and bacteria form symbioses with plants by colonising their roots. In this paper, however, saprotrophic microorganisms that live as decomposers in the soil will be the exclusive focus. The relative contribution of fungi and bacteria to the community varies between different soils in response to environmental factors (Strickland and Rousk, 2010), with fungi often dominating in terms of biomass (Joergensen and Wichern, 2008). It is generally assumed that fungi and bacteria differ with regards to the type of organic substrate they use. Bacteria are believed to mostly use labile organic compounds, whereas fungi decompose more complex organic material such as lignin and celluloses (Wardle et al., 2004; De Graaff et al., 2010; Garcia-Pausas and Paterson, 2011; Kirchman, 2012).

Total microbial biomass present in soil can be measured as extractable C, N or P following chloroform fumigation (Joergensen et al., 2011). Other methods used to estimate microbial biomass include microscopic techniques and substrate induced respiration (SIR) after addition of glucose (Anderson and Domsch, 1978). The relative contribution of bacteria and fungi to SIR can be assessed by

selective inhibition (Anderson and Domsch, 1975). However, it has been questioned whether selective inhibition is a reliable technique to distinguish between fungal and bacterial contribution to respiration. Different efficiencies of inhibitory substances and non-target effects of bactericides or fungicides can distort results (Velvis, 1997; Rousk et al., 2009). Phospholipid fatty acid analysis (PLFA), the main components of cell membranes, can be used to estimate microbial biomass and to distinguish between crude phylogenetic groups (Frostegård et al., 1993; Joergensen and Wichern, 2008). Another frequently used way to measure biomass is to measure the ATP content of soil samples (e.g. Jenkinson and Ladd, 1981; Ciardi and Nannipieri, 1990). Fungal biomass can also be measured as the amount of ergosterol, a component of fungal cell membranes (Djajakirana et al., 1996). One point of concern that has not been systematically assessed to date is the potential for soil salinity to affect the extraction efficiencies of biomarkers in soil. If this is a concern, its magnitude is likely to be more pronounced for e.g. chloroform fumigation extraction C extractions, which relies on the efficiency of an aqueous salt extraction rather than e.g. the chloroform-phenol based extraction of DNA or RNA or a lipid extraction using e.g. a Bligh and Dyer solution (Bligh and Dyer, 1959).

The microbial biomass present in the soil is the balance of a number of processes (Kirchman, 2012), including growth rate and death rate (Blagodatsky and Richter, 1998), and level of predation (Clarholm, 1981, 1985; Cotner et al., 1997). Decreased growth might not immediately lead to an observable reduction of biomass, depending on the biomass turnover rate in the soil (Bárcenas-Moreno et al., 2011). Moreover, the status of the estimated microbial biomass is completely unknown in soil. Recent attempts have suggested that active microorganisms only make up 0.1—2% and rarely exceed 5% of the total microbial biomass, while potentially active microorganisms that can quickly become activated after substrate addition comprise 10—60% (Blagodatskaya and Kuzyakov, 2013). However, the assigned status in these assessments, defining discrete levels of activity (active, vs potentially active, vs dormant, vs dead) is a simplification that risks omitting too much nuance to be useful; we know from studies of pure cultures (e.g. Monod, 1949) that the activity levels of microbial populations form a continuum of an infinite number of possible levels between zero and max. Clearly, estimations of the activity of the microbial community can therefore not be made based solely on biomass measurements.

A parameter that is often used to measure microbial activity is respiration, i.e. the amount of CO_2 that is released from a soil sample (e.g. Johnson and Guenzi, 1963; Laura, 1974; Chowdhury et al., 2011a, 2011b; Yan and Marschner, 2013a), corresponding to the rate of degradation of organic material. An increase of respiration could also be a result of stress due to changes in environmental conditions (Wardle and Ghani, 1995; Mamilov and Dilly, 2002). Respiration is often limited by substrate availability, so in the case of salinity, increased desorption of organic compounds could increase substrate availability and thus respiration (Mavi et al., 2012).

Substrate taken up by microorganisms can be incorporated into biomass or respired as CO_2. How the microbial community partitions substrate C into growth or respiration depends on the microbial C-use efficiency (analogous to other terms used in various reports, including microbial growth efficiency (MGE), growth yield efficiency, and substrate use efficiency). The microbial C-use efficiency is the amount of new biomass C produced per unit substrate C metabolised and determines the amount of C that will be lost from the soil as CO_2 (Six et al., 2006). Most studies measuring microbial C-use efficiency have been performed in aquatic systems (Cole et al., 1988; del Giorgio and Cole, 1998), whereas there is a lack of information on this parameter in soils due to a lack of useful

methods to determine growth rates (Rousk and Bååth, 2011). One measure that has been proposed to provide a measure for the microbial C-use efficiency is the microbial metabolic quotient (qCO_2) (Anderson and Domsch, 1993; Wardle and Ghani, 1995). The qCO_2 is calculated as the ratio of respiration to biomass. High values of qCO_2 are taken as a sign of low microbial efficiency and are assumed to be indicators of stress. This implicitly makes the assumption that biomass corresponds to the microbial C used for growth. This is a flawed assumption (see discussion above). Microbial growth rates are frequently offset from biomass estimates (Rousk and Bååth, 2011), and the latter are thus uninformative about the amount of C used for microbial anabolism. Moreover, if the biomass specific respiration (a better representation of what the parameter qCO_2 reflects) can be informative about anything, which part of the biomass should be included (active, potentially active, dormant or dead, sensu Blagodatskaya and Kuzyakov, 2013)?

Knowing the actual microbial growth rate is of crucial importance, because it provides information about the rate at which ecosystem processes happen. For instance, nitrification rates are significantly positively correlated with microbial growth rates (Teira et al., 2011), while in marine sediments the bacterial growth rate correlates well with the organic matter mineralization rate (Bastviken et al., 2003). Leaf litter decomposition was found to be related to fungal growth rates rather than fungal biomass (Suberkropp, 2001; Baldy et al., 2002). Methods that have been frequently used to estimate bacterial growth have included the incorporation of ^{3}H-labelled thymidine or leucine into bacterial macromolecules (Kirchman, 2001). Thymidine, one of the four nucleotides in DNA, is used to trace DNA synthesis whereas leucine, an amino acid, is used to trace protein synthesis. Since growing cells must synthesise more DNA and more protein, the amount of incorporated labelled thymidine and leucine corresponds to growth rate (Kirchman, 2001; Rousk and Bååth, 2011). Following a similar principle, by estimating the synthesis rate of lipids, fungal growth can be measured by incorporation of ^{14}C-labelled acetate into ergosterol (Rousk and Bååth, 2011). The discussed methods to estimate bacterial and fungal growth rates from rates of biosynthesis naturally rely on the quality of conversion factors to convert e.g. incorporated leucine or ergosterol to units of bacterial or fungal biomass, respectively. Such conversion factors are variable in natural communities of both bacteria (Cole et al., 1988) and fungi (Ruzicka et al., 2000; Joergensen and Wichern, 2008), and universally applicable conversion factors are probably not possible to achieve. Thus, it is important that corroborative methods are redundantly used to estimate rates of microbial growth. Other approaches to estimate microbial growth rates have included e.g. the tracking of isotopic C into microbial lipids (e.g. Arao, 1999) and DNA (Dumont and Murrell, 2005), and estimating growth rates from rates of immobilisation of nutrients (Hart et al., 1994; Bengtson et al., 2012).

The breakdown of OM by microorganisms happens through depolymerisation and oxidation of organic molecules. Extracellular enzymes catalyse the decomposition steps necessary for the breakdown of OM into units small enough to be taken up by microorganisms. Active microbes must allocate a certain minimum of C to extracellular enzyme production in order to prevent starvation (Schimel and Weintraub, 2003). Enzyme activity is therefore often measured to assess microbial activity. Assays that measure enzyme activity usually test the potential activity under substrate saturation, which is a condition very far from that normally encountered in the substrate starved soil environment (Hobbie and Hobbie, 2013). During enzyme analyses conditions such as temperature or pH can either be optimized or kept close to natural conditions (German et al., 2011, 2012), which leads to a considerable difference in results (Burns et al., 2013). Immobilised enzymes adsorbed to soil

colloids can also remain active in soils even under conditions that are unfavourable for microbial activity (Nannipieri, 2006). As a consequence, extracellular enzyme activities could be over-estimated. Current methods to estimate enzyme activities at satu-rated substrate levels and optimised conditions thus do not estimate *in situ* activities, but rather the abundance of enzymes extractable from soil, which should be largely determined by the size of the soil microbial community.

2.3. Impact of soil salinity on microbial community function and composition

2.3.1. Microbial biomass

In salt-affected soils, microbial biomass is often found to be low (Batra and Manna, 1997; Pankhurst et al., 2001; Rietz and Haynes, 2003; Yuan et al., 2007), usually coinciding with a low overall SOM content. Therefore low microbial biomass may not only be the result of a direct negative effect of salinity on mi-croorganisms, but could also be caused by a reduced input of OM

due to sparse plant growth in saline soils. One means to facilitate the sensitivity to detect direct salt effects is to express microbial biomass per SOM rather than per g soil. The percentage of mi-crobial biomass of the total SOM sometimes decreases in soils with higher salinity, suggesting that a direct negative effect on soil microbial biomass exists (Rietz and Haynes, 2003; Sardinha et al., 2003). One means to specifically test for the direct effect of salt is to estimate changes in tolerance to specific substances (see Section 2.3.5).

Reported biomass measurements from a selection of published papers were compiled and plotted against electrical conductivity (EC) (Fig. 1). Included were relevant studies found in Web of Science selected from the results generated from the search string "TOP-IC:((salt OR salinity) AND microb* AND soil)" used in ISI Web of Science Core Collection (Thomson Reuters, July 29th 2014). This search string found 1540 studies. From the search results, a sub-group of 45 relevant studies was formed, of which studies were selected that did not perform extensive manipulations of other factors than salinity (e.g. in studies where salinity and

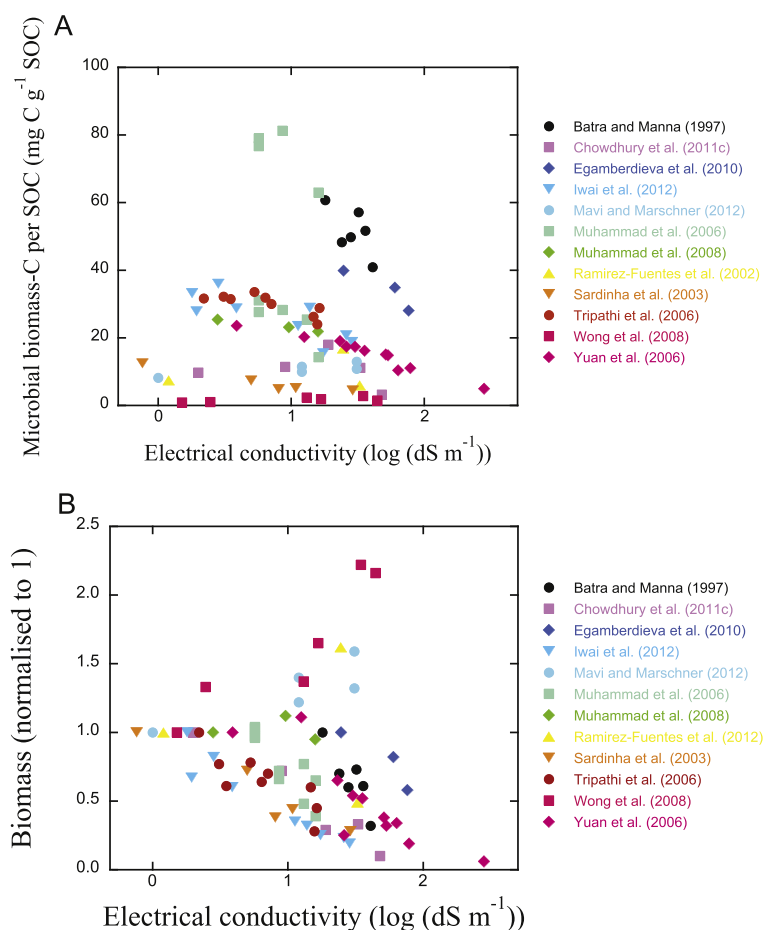

Fig. 1. Compilation of (A) published soil organic carbon (SOC) specific microbial biomass measurements and (B) relative biomass normalised to the biomass in the control treatment or in the lowest salinity sample included in the study, plotted against the electrical conductivity (in a paste; EC$_e$). Measurements were obtained by chloroform fumigation-extraction assessments of microbial C.

drying–rewetting treatments were used in a factorial design, only the salinity treatments to the control level of the drying–rewetting treatment were included; Chowdhury et al., 2011c; Mavi and Marschner, 2012) and included information on microbial biomass C, soil organic carbon (SOC), EC and soil clay content, as well as relatively wide and comprehensive ranges of salinity with at least three salinity levels. Thus, this data compilation does not claim to be exhaustive, but it can offer valuable suggestions. An overview of the datasets from the 21 selected studies is presented in Table 1. We used two methods to compensate for differences in SOM content between soils and to tease out direct salt effects: (i) microbial biomass C was calculated per soil organic carbon (SOC) (Fig. 1A), and (ii) biomass values were normalized to the biomass value measured in the sample with the lowest salinity in studies looking at field salinity gradients, or to the control treatment in studies that experimentally manipulated salinity (Fig. 1B). EC was used as a salinity metric, since this was the most common way to present salinity levels in the subgroup of relevant studies. Some authors reported EC as that in a saturated paste (i.e. extract from a water-saturated soil) (EC_e), while others reported it as that measured in a 1:5 soil:water (w:V) suspension ($EC_{1:5}$). In order to be able to combine the results into a consensus analysis, $EC_{1:5}$ values were converted to EC_e values using the following relationship (Rengasamy, 2006a):

$$EC_e = (14 - 0.13 \times [\text{clay content (\%)}]) \times EC_{1:5} \qquad (1)$$

The microbial biomass-C, expressed per g SOC (Fig. 1A) or normalised to low or no salt levels (Fig. 1B), did not show any discernable systematic relationships with soil salinity. In the scatter of data-points, some observed a decrease in microbial biomass with increasing salinity (Nelson et al., 1996; Sardinha et al., 2003; Muhammad et al., 2006; Yuan et al., 2007). For instance, Sardinha et al. (2003) found a decrease in microbial biomass along a natural salinity gradient from around 570 µg C g^{-1} to 160 µg C g^{-1}. Muhammad et al. (2006) observed a decrease from approximately 190 µg C g^{-1} to 80 µg C g^{-1}. In contrast, Wong et al. (2008) reported a considerable increase by more than 100% in total microbial biomass from around 500 µg C g^{-1} soil to around 1100 µg C g^{-1} soil with increasing salinity after applying treatments of different salinity levels to a non-saline soil and incubating for 84 days. Mavi and Marschner (2012) also observed a slight increase in microbial biomass with salinity from 93 µg C g^{-1} to 148 µg C g^{-1}, while Chowdhury et al. (2011c) found the highest microbial biomass at intermediate salinities.

While the use of the relationship described in Eq. (1) to render literature data comparable and synthesizable is a gross simplification of rather complex phenomena, it has been used extensively in soil microbial ecology research (Chowdhury et al., 2011a,b,c; Mavi et al., 2012; Elmajdoub and Marschner, 2013; Yan and Marschner, 2013a,b). To date this relationship has only been thoroughly validated for Australian soils (the listed studies), and its applicability in other soil types has yet to receive systematic study. Thus, we have here used it as a tentative first approximation to be validated by

Table 1
Overview over the studies included in the data compilation of salinity effects on biomass and respiration. Given are the duration of the study, the number of different salinity levels that were included in the study, whether soil salinity was natural or manipulated in the lab and whether soil samples were amended with an additional organic substrate source.

Study	Duration (d)	Number of salinity levels	Natural/manipulated salinity[b]	Organic substrate additions	Fig.[c]
Batra and Manna, 1997 [d]	na[a]	6	Natural	None	1
Baumann and Marschner 2013 [e]	59	3	Manipulated	Wheat straw	2
Chowdhury et al., 2011c [f]	15	5	Natural	Pea straw	1, 2
Egamberdieva et al., 2010 [g]	na[a]	3	Natural	None	1
Garcia and Hernandez 1996	58	13	Manipulated	None	2
Iwai et al., 2012 [h]	1	9	Natural	None	1, 2
Laura 1974	30	13	Manipulated	Leaves	2
Mavi and Marschner 2012 [i]	51	5	Manipulated	Wheat straw	1, 2
Mavi and Marschner 2013 [j]	30	5	Manipulated	Wheat straw	2
Mavi et al., 2012	42	4	Manipulated	Wheat straw	2
Muhammad et al., 2006	58	10	Natural	None/wheat straw	1, 2
Muhammad et al., 2008 [k]	7	3	Natural	None	1, 2
Pathak and Rao 1998	90	5	Manipulated	Plant material	2
Ramírez-Fuentes et al., 2002	140	3	Natural	Glucose	1, 2
Sardinha et al., 2003	4	5	Natural	None	1, 2
Saviozzi et al., 2011	40	4	Manipulated	None	2
Setia et al., 2011a [l]	7	6	Manipulated	Wheat straw	2
Tripathi et al., 2006	10	9	Natural	None	1, 2
Wichern et al., 2006 [m]	47	3	Manipulated	None	1
Wong et al., 2008 [n]	84	6	Manipulated	None	1, 2
Yuan et al., 2007	10	11	Natural	None	1, 2

[a] Not available.
[b] Natural salinity refers to studies that used samples from natural, environmental salinity gradients, while manipulated salinity refers to studies that experimentally manipulated soil salinity in a laboratory setting.
[c] Denotes which figures include data from the respective studies.
[d] The study looked at soil layers in different depths, but only data from the 0–15 cm layer were used in this data compilation.
[e] The authors looked at effects of drying–rewetting in saline soils, but here only data from the constantly moist treatment were included.
[f] The authors looked at effects of drying–rewetting in saline soils, but here only data from the constantly moist treatment were included.
[g] The authors grouped 12 soils into 3 groups (weakly, moderately and highly saline).
[h] The study looked at the dry and wet season in a subtropical savanna climate, but only dry season data were used.
[i] The authors looked at effects of drying–rewetting in saline and sodic soils, but here only data from the constantly moist treatment were included.
[j] In the study different types of plant material were added to the soil, but only the wheat straw treatment were included in this data compilation.
[k] 29 soils were grouped into three groups by the authors (saline, sodic and saline-sodic).
[l] Respiration and biomass data for each salinity level are the mean values of 4 soils of different textures (sandy loam, sandy clay loam, loamy sand, clay).
[m] The study also includes a treatment with maize addition, which showed a similar effect of salinity, but was not included in the data compilation.
[n] The authors looked at soil layers in different depths, but only data from the 0–5 cm layer were used in this data compilation.

available data. The lack of observable systematic trends between soil microbial biomass and estimated salinity (EC_e) has two possible explanations. Either the metrics do not capture the intended dependent and independent variables well, or there is really a lack of a relationship. If the simplified estimate of the conductivity in a paste (Eq. (1)) was too simplistic to usefully generalize this could have led to flawed metrics. The two possible explanations will be revisited in the section on respiration, below.

2.3.2. Microbial respiration

Respiration is one of the most studied parameters describing the response of the microbial activity to environmental factors, including salinity. It is the most direct measure of the overall microbial contribution to the C cycling. Respiration measurements were collected from published studies using the same search string as above (Section 2.3.1). We selected 17 studies that included data on respiration, EC and clay content, and those that covered at least a range of three salinity levels. The data compilation used results obtained for cumulative respiration at the end of the study period (ranging from 4 to 90 days; Table 1). Respiration measurements differed by several orders of magnitude between studies. To isolate the effect by salinity, values were normalized to the respiration measured in the sample with the lowest salinity in studies looking at field salinity gradients, or to the control treatment in studies that experimentally manipulated salinity (Fig. 2). The normalized values were calculated by dividing the respiration values measured at a certain salinity level by the control values (i.e. normalised to unity). A logistic consensus curve was fitted to the data points using the model:

$$y = c/[1 + e^{b(x - a)}] \qquad (2)$$

where y is the normalized respiration rate, x is the logarithm of EC_e, a is the logarithmic value of the electrical conductivity resulting in 50% inhibition of respiration (log(EC_{50})), c is the respiration rate in the control and b is a fitted parameter (slope) indicating the inhibition rate (Rousk et al., 2011b).

The consensus curve fitted to the data compilation shows a consistent decline of respiration with higher salinity level ($R^2 = 0.24$; $n = 131$; $P < 0.01$). While the biomass compilation (Fig. 1) allows for the possibility that our attempt to synthesize the data with the relationship of Eq. (1) did not work well (see previous section), the clear relationship between normalised respiration and

estimated salinity vindicated the used assumptions, and suggests that the gross simplification used in Eq. (1) may be useful.

Respiration in soils from natural salinity gradients is in most cases significantly negatively correlated with salinity (e.g. Sardinha et al., 2003; Yuan et al., 2007; Muhammad et al., 2008). However, it is difficult to tease out direct salt effects on the microbial community from indirect effects due to reduced input of plant material (also see Section 2.3.5. where tolerance could provide a means to distinguish indirect from direct effects of salt). Soils with a lower SOM have a lower availability of C substrate (Rietz and Haynes, 2003; Sardinha et al., 2003) causing lower respiration rates. Since C is the primary determinant of the soil microbial community size (Wardle, 1992) one possibility to avoid confounding direct with indirect salt effects is to express the measured respiration per microbial biomass (as an index for a long-term C availability) or per unit SOM. Another possibility is to experimentally expose one soil to several levels of salinity. In accordance with the findings based on naturally saline soils, respiration also declined in originally non-saline soils that were incubated at different salinities in the laboratory (e.g. Laura, 1974; Garcia and Hernandez, 1996). This suggests a strong negative direct impact of salinity on microbial activity and the decomposition of organic matter on the existing microbial community, which is not yet adapted to higher levels of salinity. It should also be possible to detect direct effects of salinity by screening for increased community tolerance to salt (see Section 2.3.5.). If salinity exerts an ecologically relevant effect on the community, more salt-tolerant organisms should be favoured, inducing a shift towards a more tolerant community.

A decrease in respiration has been observed both in studies that looked at basal respiration and studies that added organic matter as an additional C source (Setia et al., 2011a,b; data used in Fig. 1 are mean values of the different soil types studied in those reports). Organic matter amendment is often applied to saline soils in order to improve plant growth and has been suggested as a way to stimulate soil microbial activity in saline soils (Setia and Marschner, 2013), which could be suggestive for general mitigation against salinization effects. Setia et al. (2011b) sampled soils covering a range of different salinities and incubated them in the laboratory with and without amendment of wheat residue. While respiration was higher in the amended soils, in both treatments salinity had a strong negative impact on soil respiration. Conversely, Muhammad et al. (2006) observed a reduction of the negative impact of salinity on respiration in soils amended with plant residue compared to

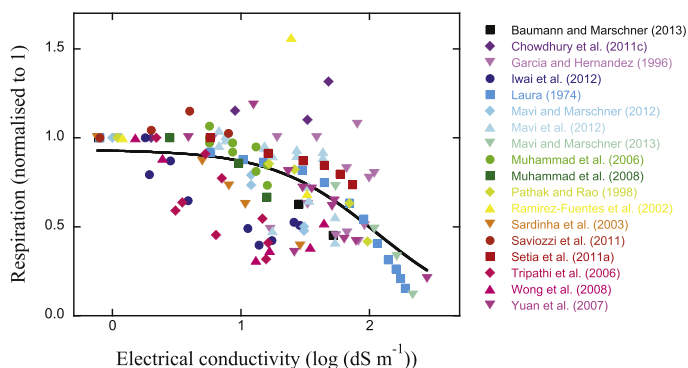**Fig. 2.** Respiration measurements collected from the literature plotted against the electrical conductivity (in a paste EC_e). Values represent cumulative respiration at the end of the study period. Results were normalised to the respiration in the control treatment or in the lowest salinity sample included in the study. A logistic model was used to fit a consensus curve to the data points ($R^2 = 0.24$ $n = 109$; $P < 0.01$).

soils without amendment. Respiration in residue-amended soils was considerably higher than in soils without amendment and not negatively affected by salinity. Also the amount of recovered straw at the end of the experiment did not differ between salinity treatments, indicating that OM decomposition was not negatively impacted by salinity. It is possible that increased substrate availability following OM amendment would yield surplus resources that microorganisms could invest in osmolyte production (Schimel et al., 2007).

The type of salt used (e.g. sodic Na^+-salts vs. Ca^{2+}-salts) will also influence results obtained by different studies. For instance, Nelson et al. (1996) found that in soils adjusted to different levels of salinity and sodicity, respiration decreased with higher salinity but increased with increasing sodicity. Saviozzi et al. (2011) incubated originally non-saline soil at different concentrations of NaCl and observed an increase in respiration compared to the control, even though microbial biomass (measured as ATP) decreased. A mechanism that could explain an increase of respiration when sodicity is increased, is the dispersion of soil aggregates and the higher availability of organic substrates at increasing Na^+ concentrations.

2.3.3. Microbial growth and C-use efficiencies

In contrast to respiration and biomass, information on the effect of soil salt concentration on the growth rate, i.e. the rate of biomass production, is scarce (Rousk and Bååth, 2011). One study by Rousk et al. (2011b) investigated the relationship between *in situ* salt concentrations along with the microbial salt tolerance in several saline soils, and found that high salt concentrations could inhibit growth by more than 90%. However, although reports are lacking from terrestrial environments, a large body of work has been done in aquatic environments. In most of these reports, the effect of salinity on growth has been studied using incorporation of ^3H-labelled leucine or thymidine. For instance, growth rates of heterotrophic prokaryotes in saltern systems, a series of interconnected ponds used for salt making, were found to decrease with increasing salinity (Pedrós-Alió et al., 2000; Gasol et al., 2004). Schultz et al. (2003) observed an upstream increase in specific growth rate (growth per unit biomass) that was correlated with declining salinity along a salinity gradient in the York River estuary in Virginia. The opposite pattern was found by Troussellier et al. (2002) who recorded an increase in specific growth rates in the Rhone estuary towards the river mouth. In the Rhone estuary cell abundance decreased strongly towards the river mouth, while thymidine and leucine incorporation rates remained roughly the same. Both studies observed an inverse relationship between cell abundance and growth rate. In a study by Bouvier and del Giorgio (2002) bacterial production and bacterial growth efficiency was lowest in the middle estuary within the turbidity maximum region, where freshwater and saltwater mix and salinity is more variable. Whether production and growth efficiency were higher in freshwater or marine samples was seasonally dependent. During high rainfall in spring and autumn production was higher at upstream stations with lower salinity, but during May, a month with low rainfall, bacterial production was higher at downstream stations after the turbidity maximum region.

These few examples of studies that specifically investigated the effects of salinity gradients in aquatic systems on bacterial production serve as an illustration of the rich body of work on salt available from aquatic systems. This accumulated knowledge should be used to guide hypothetical deductive work in terrestrial environments. In addition, if salinization of soils will lead to a shift from fungal to bacterial dominance (e.g. Pankhurst et al., 2001), this would further emphasize the relevance of knowing how bacteria are affected by salt; information that abound for aquatic systems.

There is currently a lack of information on how salinity affects the soil microbial C budget, since very few studies on salinity effects in soil have included growth. In addition to merely being an alterative, sensitive end-point of assessing responses of microbial communities to salt, relating the anabolic and catabolic use of C should also add predictive value for long term C stock development. While most of the C that forms SOM initially was sourced from plants, a majority of the SOM that accumulates in soil is composed of microbial rather than plant derived C (Kögel-Knabner et al., 2008; Marschner et al., 2008; Kleber, 2010; Malik and Gleixner, 2013). Thus, only the fraction of C that is allocated to growth, rather than catabolic processes, has the potential to influence SOM stocks (Miltner et al., 2012). To further our understanding of microbial C dynamics in saline soils, a comparative analysis of the sensitivity of different microbial processes, contrasting anabolic to catabolic, to increasing salt concentrations is required. Since the SOM content of soils is a determinant of soil fertility, this would impact plant growth and agricultural crop production and as a consequence affect the quantity and quality of plant-derived below-ground and above-ground biomass input to the soil. Moreover, an explicit consideration of feedbacks between above- and below-ground effects by salinization, currently an active research area for other factors in soil (e.g. Bardgett et al., 2013) has yet to be conducted.

2.3.4. Microbial enzyme activities

Generally, extracellular enzyme activity is lower in naturally saline soils than in non-saline soils (Batra and Manna, 1997; Rietz and Haynes, 2003; Ghollarata and Raiesi, 2007). Enzyme activity assays usually measure potential activity at substrate saturation (Burns et al., 2013). Results are likely to be influenced by lower organic matter and microbial biomass content in saline soils. In soils with manipulated salinity enzyme activity was also found to decrease with increasing salinity, indicating that there is a direct negative effect of salinity on enzyme activity (Frankenberger and Bingham, 1982; Garcia and Hernandez, 1996; Pathak and Rao, 1998; Saviozzi et al., 2011). Pathak and Rao (1998) found that addition of organic matter stimulated enzyme activity by increasing microbial biomass, but it was still negatively correlated with salt concentration.

In addition to lower biomass, another mechanism often used to explain lowered enzyme activity is that microbial communities faced with low osmotic potentials allocate less resource to protein production and release fewer proteins into the medium. In addition, high salt concentrations also denature proteins and reduce their solubility and thus lower enzyme activity (Frankenberger and Bingham, 1982). High salt concentration can also lead to the dispersion of colloids, leaving extracellular enzymes more susceptible to decomposition (Garcia and Hernandez, 1996).

One problem with using enzyme activity as an indicator of microbial activity is that results can vary considerably between different soils and different enzymes. For example, Frankenberger and Bingham (1982) found dehydrogenase activity more strongly negatively affected by salinity than hydrolase activity, whereas Garcia and Hernandez (1996) obtained the opposite result using a different soil. In fact, in the latter study oxidoreductase activity even increased at higher salinity levels. Saviozzi et al. (2011) found that while protease and dehydrogenase were inhibited with increasing salinity, amylase and phosphatase showed no correlation with salinity and catalase was even slightly stimulated. It remains elusive to identify common general patterns from the assessments of extracellular enzyme activities in saline soils. This suggests that other metrics may be preferable to assess microbial functioning in saline soils. Alternatives include e.g. DNA/RNA based molecular methods (see section 2.3.5).

2.3.5. Fungal-to-bacterial balance and microbial community structure

A number of studies have looked at the change of the fungi-to-bacteria-ratio in response to increasing salinity. Fungi are thought to be more resistant to low water potentials and osmotic stress than soil bacteria (Griffin, 1972). For instance they have been found to be better able to cope with high substrate loading rates of low molecular weight C compounds than bacteria (Griffiths et al., 1999; Reischke et al., 2014). In naturally saline soils, however, an increasing importance of fungi at higher salt concentrations has not been confirmed. Sardinha et al. (2003) found a strong decrease in the ratio of the fungal biomarker ergosterol to biomass C with environmental salt concentrations. In the least saline site fungi made up 90% of the microbial biomass, but only 17% at the most saline site. Also Pankhurst et al. (2001) reported a lower fungal-to-bacteria ratio in salt-affected soils. How much of this shift towards a bacteria-dominated community in saline soils is due to a direct negative effect of salinity on fungi is unclear. An alternative explanation that should be considered is that salt effects on the plant community could be influential, and need to be taken into account to begin to assign the effect to an indirect or a direct consequence of salt.

Wichern et al. (2006) incubated a naturally saline and a non-saline soil at different levels of salinity with added plant residue. Before the start of the experiment the saline soil had a far lower fungal-to-bacteria ratio. During the incubation experiment the authors observed an increase of the ergosterol-to-microbial biomass ratio following the addition of plant residue. A weak effect of salinity on ergosterol-to-microbial biomass ratio could also be observed, showing a slight increase with salinity. An explanation for the observed increase in ergosterol could be an accumulation of ergosterol in more salt-tolerant fungi, since fungi grown at higher salt concentrations have been found to have a higher ergosterol content in their cell membranes (Hosono, 1992). Also ergosterol could have originated from an accumulation of fungal necromass, since ergosterol can be slow to turn over in both soil and litter samples (Mille-Lindblom et al., 2004; Zhao and Brookes, 2005). Chowdhury et al. (2011a) also incubated soil samples at different salinities with added organic matter, but reported a decrease in the proportion of fungal phospholipid fatty acids (PLFAs) with higher salinity.

The emergence of high-throughput sequencing techniques over the last decade makes it possible to obtain a more comprehensive and in-depth picture of the microbial community composition, but they have rarely been applied to saline soils. In a global meta-analysis comparing bacterial community composition of a wide range of different environmental samples from both aquatic and terrestrial habitats, salinity was found to be the major environmental determinant of bacterial community composition in aquatic environments, more important than even pH or temperature (Lozupone and Knight, 2007). However, saline soils were not included in the report, highlighting the lack of knowledge on the effect of salinity on terrestrial microbial community structure. Surprisingly no studies of bacterial community composition along gradients in soil salinity using up-to-date molecular techniques seem to exist. There seems to be no methodological reason to explain this lack of information, since microbial communities in marine sediments with salinities comparable to those in saline soils have been successfully studied (e.g. Wang et al., 2012; Hamdan et al., 2013). Community composition along salinity gradients in estuarine or marine environments has received a lot of attention (e.g. Fortunato et al., 2012; Campbell and Kirchman, 2013). In these studies freshwater and marine ecosystems were found to harbour very distinct communities, with a rapid change in community composition at a certain salinity threshold. In contrast, information on community shifts along soil salinity gradients is currently very rudimentary, but studies on fungi and archaea indicate that salinity has a strong impact on shaping microbial communities also in soil. For instance, Walsh et al. (2005) reported a shift in archaeal community composition along a soil salinity gradient and changes in fungal composition along a marshland salinity gradient have also been observed (Mohamed and Martiny, 2011).

By obtaining more information on the microbial community composition it could be possible to link functional responses to salt to changes in community structure. It has been suggested that more diverse communities have higher functional stability (Griffiths et al., 2000). Certain soil functions controlled by microorganisms, including e.g. ammonia oxidation, can be impaired in communities with low diversity (Baumann et al., 2013; Philippot et al., 2013). However, these are topics that still await systematic and exhaustive evaluation in soil, as reports of up-to-date molecular characterisations of soil microbial community responses to salt have yet to be published.

2.3.6. Microbial tolerance assessments

One way to detect direct salt effects on the microbial community is to draw upon the concept of pollution-induced community tolerance (PICT), which is used in ecotoxicology to detect the effects of contaminants on communities (Blanck, 2002; Boivin et al., 2002). Community tolerance is an aggregate measurement of the ability of a community to withstand a contaminant. As such, it is a more comprehensive measure than tolerance on the cell level. When a community is exposed to a contaminant, organisms that can tolerate the contaminant are favoured, while the abundance of more sensitive species will decrease. This selection for more tolerant organisms will shift the community composition towards a community with a higher tolerance. The changed community tolerance in response to a toxicant is called PICT (Fig. 3).

In PICT detection experiments community tolerance is quantified as the concentration of a toxicant necessary to inhibit activity or induce death to a certain level (usually 50%). The tolerance of communities pre-exposed to the toxicant is compared to the tolerance of previously unaffected communities (e.g. Aldén Demoling and Bååth, 2008; Fig. 3). If a specific contaminant had an ecologically relevant impact on shaping the community in the past, pre-exposed communities should have a higher community tolerance. Pollution-induced community tolerance in soil microbial communities has been demonstrated for a wide range of contaminants, including antibiotics (Aldén Demoling and Bååth, 2008; Aldén Demoling et al., 2009; Brandt et al., 2009), heavy metals

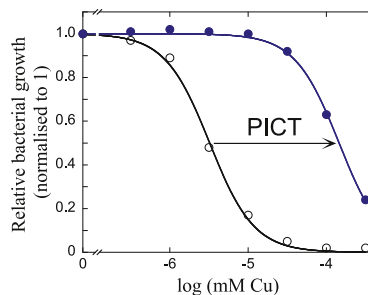**Fig. 3.** Example of a PICT-assay of copper (Cu)-tolerance of the bacterial community. Open circles show the tolerance of a sensitive community (the control treatment), and filled circles that of a more Cu-tolerant community (as induced by exposure to Cu). The difference between the curves is a measure of the Pollution Induced Community Tolerance (PICT). Redrawn from data from Fernández-Calviño et al. (2011a).

(Stefanowicz et al., 2009; Fernández-Calviño et al., 2011a; Berg et al., 2012) and herbicides (Zabaloy et al., 2010) among others.

Similarly to PICT assessments with the aim to detect effects of toxicants, comparing tolerance with *in situ* conditions can identify environmental factors that play an ecologically significant role in shaping the community. In this vein soil pH tolerance for bacterial growth was found to track field pH values, suggesting that pH exerts a selective pressure on the bacterial soil community (Fernández-Calviño and Bååth, 2010; Fernández-Calviño et al., 2011b; Pettersson and Bååth, 2013). With regards to soil salinity, it could likewise be hypothesized that microbial communities from saline soils will be less affected by high salt concentrations than communities from non-saline soils, since they should harbour a more tolerant community.

Increased tolerance to salt in pre-exposed populations has been documented on the level of bacterial strains in culture (Killham, 1994; Trabelsi et al., 2010). On the community level, Wichern et al. (2006) found indications for salt adaptation in a microbial community from a saline soils. Specifically, at higher salt additions the community from a naturally saline soil was better able to decompose added plant material than a community from a non-saline soil, even though at low salinities they were less effective decomposers.

Conversely, in an experiment applying the PICT methodology, Rousk et al. (2011b) found no statistically significant relationship between *in situ* salt concentration and community tolerance, which indicates that other factors than salt must have been more important in shaping the respective communities. Only after incubating the soils for a month with added plant material as organic substrate was there a weak relationship between soil salinity and salt tolerance. Baumann and Marschner (2013) reported that microbial communities extracted from saline soils had higher resistance to drying rewetting cycles, which was taken to suggest that tolerance to osmotic stress had been induced by salt exposure. Studies by both Asghar et al. (2012) and Yan and Marschner (2012) found no relationship between soil salt concentrations and tolerance with regards to respiration. In both studies respiration decreased with increasing salinity, irrespective of original salinity.

Another interesting question is how the community responds after a contaminant or stressor has been removed. Since tolerance mechanisms usually pose an energetic burden for the organism (Kashian et al., 2007), it seems likely that after the removal of the specific stressor, tolerant species or strains would have a competitive disadvantage and overall community tolerance would decrease again. Currently only a few studies have looked at the development of community tolerance after stress removal. Aldén Demoling and Bååth (2008) observed a quick return of community tolerance to pre-exposure levels after the removal of the antibiotic tylosin. In a different study, Díaz-Raviña and Bååth (2001) found that metal-tolerant communities quickly lost most (70—90%) of their acquired tolerance in the first week after the removal of the contaminant metal. After that no further changes in community tolerance happened, and the communities retained a low level of metal-tolerance even twelve months after the removal of metal contamination.

The specific cause for the inhibition of microbial communities and processes by salt is another aspect that can be assessed with enhanced resolution using tolerance determinations. While the unified response of respiration to elevated electrical conductivity across studies (despite sometimes being exposed to a natural mixture of salts, sometime to e.g. pure NaCl) suggests that the dominant factor is the ionic strength (which is indexed by the EC_e), it is possible that the toxic effect of specific salt ions can also be more dominant (Kinraide, 1999). Additionally, soil salinization is predominantly an agricultural problem in arid soils and is often

related to irrigation and water supply. In these soils, a parallel factor of high impact for microbial functioning is drought and especially cycling drought. That one of the central problems in soil microbiology has become the respiratory pulse that stems from rewetting dry soils (Birch, 1958; Göransson et al., 2013; Warren, 2014) highlights the importance of this associated factor. Moreover, the physiological effects of salt and of variable moisture are united in their influence on osmotic potentials that organisms need to resist. While some pioneering studies have been initiated to attempt to discriminate and assign cause to salinity and drying rewetting (e.g. Chowdhury et al., 2011c; Baumann and Marschner, 2013; Kakumanu and Williams, 2014) the interaction between these environmental factors is an interesting challenge yet to systematize and resolve. Establishing which factors have resulted in a change in tolerance within the microbial population, i.e. determining PICT, would provide a potential to resolve these questions (Blanck, 2002). It must be noted, however, that unlike establishing the tolerance to toxic chemicals, which have a clearly developed methodology (acute inhibition of endpoint in a dose—response curve), estimating the tolerance to drought or drying—rewetting is far from trivial. The end-point to assess in itself would be a challenge to determine (e.g. the rate of recovery, or the level of inhibition), along with the time-frame used for the determination.

2.3.7. Microbial resistance and resilience

The ability of microbial communities to withstand changes in environmental conditions can be described using the terms resistance and resilience (see recent overview in Griffiths and Philippot, 2013). Resistance describes the ability of a community to withstand a change in conditions without a change in a community metric, while resilience refers to the rate at which a community returns to its original state, once the previous environmental conditions are re-established (Allison and Martiny, 2008; Shade et al., 2012; Griffiths and Philippot, 2013). Changes in community composition can be accompanied by changes in microbial function (Fig. 4). But even if a community is neither resistant nor resilient, processes could continue at the same rate, if the community contains a high degree of functional redundancy (Allison and Martiny, 2008), i.e. one microbial taxon can replace another in carrying out a process at the same rate.

Saline soils are assumed to harbour communities that use C sources less efficiently than communities in non-saline soils (Rietz and Haynes, 2003; Wichern et al., 2006; Yuan et al., 2007). An interesting question is how these communities will develop after the salt stress has been removed and if they will revert to a community with more efficient C-use. Microbial communities subjected to high salt concentrations appear to have a high degree of resilience. Yan and Marschner (2013a) found that respiration and biomass are able to recover rapidly after leaching of naturally saline soils irrespective of the original salinity, suggesting that microbial function can recover quickly, at least in the short time-frame, after amelioration of saline soils. In another recent study the same authors increased or reduced soil salinity in naturally saline and non-saline soils over six 5-day cycles (Yan and Marschner, 2013b). Reduced at high salinity, respiration rapidly recovered if the salinity was subsequently lowered. Compared to the originally saline soils, respiration and biomass in the originally non-saline soil were higher, less affected by salinity increases and recovered more quickly after the salinity was decreased. However, the authors looked at respiration per gram soil and not related to microbial biomass, which was considerably lower in the saline soil. Berga (2013) exposed bacterial communities from freshwater rock pools to a gradient of increasing salinity and found that communities were resistant to small changes in salinity but showed a
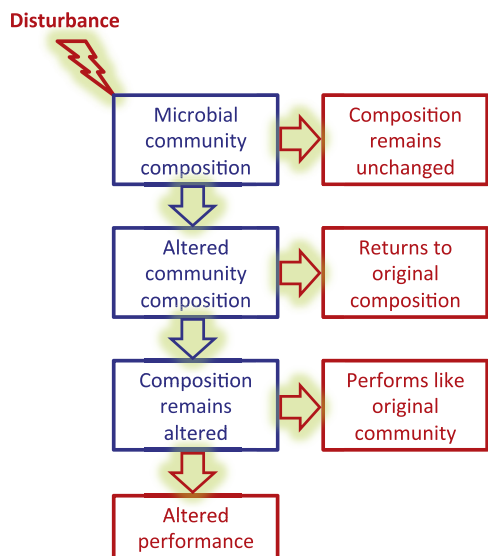
Disturbance

Fig. 4. A schematic representation of how disturbance (e.g. a change in environmental conditions) can change microbial community composition and thus microbial processes. Resistance, resilience and functional redundancy can prevent structural or functional effects of disturbance on the microbial community. Redrawn from Allison and Martiny (2008).

composition change at higher salinity values. When salinity concentrations were decreased to the starting level the bacterial community returned to its initial composition within 4 days, suggesting high resilience.

Despite the high resilience of microbial communities, historical factors in the form of past environmental conditions have been found to play a role in microbial community composition and function. Keiser et al. (2011) found that distinct soil microbial communities incubated in microcosms with the same environmental conditions remained functionally dissimilar over almost one year. Langenheder et al. (2006) also observed that the community composition of distinct microbial communities grown under identical conditions did not develop into more similar communities with regards to community composition. One important limitation of laboratory studies like these is that they take place in spatially isolated environments and do not take dispersal into account, whereas in natural aquatic ecosystems microorganisms are assumed to have high dispersal rates. In soil on the other hand, dispersal rates are slower. Therefore immigration of new organisms could supersede historical effects in aquatic ecosystems more so than in terrestrial ones. Logares et al. (2013) studied microbial community composition in Antarctic lakes that were isolated from the sea 20 000 years ago and subsequently underwent a salinity change that established a gradient ranging from freshwater to hypersaline. They found that community composition was strongly correlated with salinity, but only weakly with geographical distance, suggesting that communities were mostly structured by local environmental factors rather than dispersal. Still there are indications that over shorter timescales even in natural ecosystems, historical effects could have a persisting effect on microbial community composition. In a study on the bacterial community composition of rock pools, Langenheder and Ragnarsson (2007) found an effect of spatial

factors on community composition over small spatial scales that was not explained by the environmental factors included in the study and could reflect historical events. In another study in the same rock pool system bacterial community composition approximately one week after a rain event was better explained by past pre-rainfall salinity than current salinity (Andersson et al., 2014).

Overall, research on the effects of changing salinity on microorganisms has been more extensive in aquatic than in terrestrial systems. In most aquatic systems, such as the oceans, salinity levels are more stable than in saline soils, where salinity levels can fluctuate highly in response to rainfall, irrigation or drought events. The variations in salinity encountered by aquatic microorganisms living in estuarine environments probably come closer to what soil organisms experience (e.g. Sjösted et al., 2012). Another situation that could resemble the type of lasting salinity gradient that we can find in terrestrial environments can be found where fresh water plumes mix into marine environments (e.g. Fortunato et al., 2012). While these situations can be used to generate hypotheses of how microbial communities respond to salt in soil, to date it should be noted that it is not clear in how far microbial reactions to salinity differ between aquatic and terrestrial environments, since no study has yet compared the two.

3. Conclusions and some future research directions

3.1. Toward general patterns for respiration and microbial biomass

The response of microbial biomass and respiration to soil salinity has been assessed in a substantial number of studies to date, and we were able to compile and interrelate some of that data (Figs. 1 and 2, Table 1). The response of microbial biomass to salinity was variable (Fig. 1). Since the biomass is susceptible to a range of processes, including growth rate, death rate, and level of predation and since its status is unknown biomass has been suggested to only be very indirectly related to active processes in the soil (see Section 2.3.1). In contrast with biomass, the overall effect by salt on respiration was clearly negative (Fig. 2). This appeared to be consistent for both soil samples exposed short-term as for soil samples collected from long-term gradients (see Section 2.3.2.). This suggest that overall decomposer functioning will be impaired by salinization and that the possibility for microorganisms to adapt to high salt concentrations (i.e. the long term salt gradients) did not make the decomposer microorganisms perform better than samples exposed in the shorter term (laboratory experiments). However, a confounding factor of unknown leverage in the obtained relationships is how influential reduced plant productivity was in the samples collected from long-term gradients highlighting a research priority yet to address. It also should be noted that under field conditions soil salinity is a constantly changing factor, providing an experimental challenge for short- and long-term studies.

To date, there is a shortage of growth based assessments of microbial responses to salinization. While expectations exist about a reduced efficiency of the microbial C-use budget, there is a shortage of underlying data, and a systematic investigation of how the microbial C-use budgets are affected is lacking. We also note an alarming lack of assessments of how soil salinity can structure the soil microbial community using up-to-date molecular methods. This despite the clear evidence from aquatic microbial ecology (e.g. Lozupone and Knight, 2007), showing a potential for salt to affect microbial communities on par with that of pH. This development will also be an important addition to enable the suggested establishment of microbial biomarkers (see next section) to characterise soils and aid their management.

3.2. Tolerance to assess ecology and develop biomarkers

If salinity has a direct, ecologically relevant effect on shaping soil microbial communities, pre-exposed communities should have a higher community tolerance to salt. To date, there is limited evidence for a connection between the salinity level in soil and the microbial tolerance to salt. However, there are some indications that communities from saline soils could be better adapted to high salt concentrations than communities from non-saline soils, which warrant further investigation. Moreover, if microbial community tolerance to salt is induced that should be accompanied by systematic shifts in microbial community composition. If high-resolution molecular surveys of microbial communities in saline soils (see Section 3.1) are combined with tolerance assessments, the opportunity to develop useful biomarkers (e.g. sequences) presents itself. This could provide a useful tool that could be used to predict a soil's sensitivity to salt. This type of predictive power would of course be a useful tool for soil resource management.

3.3. Beyond environmental threats: probes in microbial ecology

Soil salinization is one of the most pressing environmental challenges to resolve in the current century (Griggs et al., 2013). In addition to this, it also highlights a useful arena where we can generate insights about the ecology of microorganisms in soil. In aquatic microbial ecology, salinity has been identified as one of the most potent environmental factors for microorganisms (Lozupone and Knight, 2007). Consequently, it is already being used as an experimental factor to investigate the fundamental ecology of microbial communities in both the laboratory (Lindström and Östman, 2011; Severin et al., 2013) and the field (Reed and Martiny, 2013). There have been some recent pioneering attempts to use salinity as an experimental probe to link microorganisms to biogeochemical functions in soil (e.g. Chowdhury et al., 2011a, b; Mavi and Marschner, 2013; Yan and Marschner, 2013b), but so far only the surface has been scratched of a reservoir of considerable potential. The well-known power of temperature (Kirschbaum, 1995; Davidson and Janssens, 2006; Bradford, 2013) and moisture (Schimel et al., 2007; Manzoni et al., 2012) to regulate microbial communities has provided avenues toward general insights into microbial ecology (Hartley et al., 2008; Placella et al., 2012; Göransson et al., 2013; Rousk et al., 2012, 2013). We foresee that equivalent possibilities exist for chemical environmental factors, including salinity. Recent use of soil pH as an experimental factor to "push" microbial communities (Rousk et al., 2010; Fernández-Calviño et al., 2011b; Pettersson and Bååth, 2013) could easily be adapted to instead use salt. Moreover, employing similar environmental factors to probe microbial communities in both soil (adding salinity as an experimental factor; as in e.g. Severin et al., 2013) and water (adding pH as an experimental factor; as in e.g. Pettersson and Bååth, 2013) would provide an interesting system comparison.

Acknowledgements

References

Aldén Demoling, L., Bååth, E., 2008. No long-term persistence of bacterial pollution-induced community tolerance in tylosin-polluted soil. Environmental Science & Technology 42, 6917—6921.

Aldén Demoling, L., Bååth, E., Greve, G., Wouterse, M., Schmitt, H., 2009. Effects of sulfamethoxazole on soil microbial communities after adding substrate. Soil Biology & Biochemistry 41, 840—848.

Allison, L.E., 1964. Salinity in relation to irrigation. Advances in Agronomy 16, 139—180.

Allison, S.D., Martiny, J.B.H., 2008. Resistance, resilience, and redundancy in microbial communities. Proceedings of the National Academy of Sciences of the United States of America 105, 11512—11519.

Anderson, J.P.E., Domsch, K.H., 1975. Measurement of bacterial and fungal contributions to respiration of selected agricultural and forest soils. Canadian Journal of Microbiology 21, 314—322.

Anderson, J.P.E., Domsch, K.H., 1978. Physiological method for quantitative measurement of microbial biomass in soils. Soil Biology & Biochemistry 10, 215—221.

Anderson, T.H., Domsch, K.H., 1993. The metabolic quotient for CO_2 (qCO_2) as a specific activity parameter to assess the effects of environmental-conditions, such as pH, on the microbial biomass of forest soils. Soil Biology & Biochemistry 25, 393—395.

Andersson, M.G.I., Berga, M., Lindström, E.S., Langenheder, S., 2014. The spatial structure of bacterial communities is influenced by historical environmental conditions. Ecology 95, 1134—1140.

Arao, T., 1999. In situ detection of changes in soil bacterial and fungal activities by measuring ^{13}C incorporation into soil phospholipid fatty acids from ^{13}C acetate. Soil Biology & Biochemistry 31, 1015—1020.

Arshad, M., 2008. Soil salinity and salinization. In: Chesworth, W. (Ed.), Encyclopedia of Soil Science Dordrecht. Springer, pp. 699—704.

Asghar, H.N., Setia, R., Marschner, P., 2012. Community composition and activity of microbes from saline soils and non-saline soils respond similarly to changes in salinity. Soil Biology & Biochemistry 47, 175—178.

Ayers, R.S., Westcot, W.D., 1976. Water Quality for Agriculture. Italy, FAO, Rome.

Bååth, E., Söderström, B., 1979. Fungal biomass and fungal immobilization of plant nutrients in Swedish coniferous forest soils. Revue D Ecologie Et De Biologie Du Sol 16, 477—489.

Bajwa, M., Josan, A., Hira, G., Singh, N., 1986. Effect of sustained saline irrigation on soil salinity and crop yields. Irrigation Science 7, 27—35.

Baldy, V., Chauvet, E., Charcosset, J.Y., Gessner, M.O., 2002. Microbial dynamics associated with leaves decomposing in the mainstem and floodplain pond of a large river. Aquatic Microbial Ecology 28, 25—36.

Bárcenas-Moreno, G., Rousk, J., Bååth, E., 2011. Fungal and bacterial recolonisation of acid and alkaline forest soils following artificial heat treatments. Soil Biology & Biochemistry 43, 1023—1033.

Bardgett, R.D., Manning, P., Morrien, E., De Vries, F.T., 2013. Hierarchical responses of plant-soil interactions to climate change: consequences for the global carbon cycle. Journal of Ecology 101, 334—343.

Bastviken, D., Olsson, M., Tranvik, L., 2003. Simultaneous measurements of organic carbon mineralization and bacterial production in oxic and anoxic lake sediments. Microbial Ecology 46, 73—82.

Batra, L., Manna, M.C., 1997. Dehydrogenase activity and microbial biomass carbon in salt-affected soils of semiarid and arid regions. Arid Soil Research and Rehabilitation 11, 295—303.

Baumann, K., Dignac, M.F., Rumpel, C., Bardoux, G., Sarr, A., Steffens, M., Maron, P.A., 2013. Soil microbial diversity affects soil organic matter decomposition in a silty grassland soil. Biogeochemistry 114, 201—212.

Baumann, K., Marschner, P., 2013. Effects of salinity on microbial tolerance to drying and rewetting. Biogeochemistry 112, 71—80.

Bengtson, P., Barker, J., Graystron, S.J., 2012. Evidence of a strong coupling between root exudation, C and N availability, and stimulated SOM decomposition caused by rhizosphere priming effects. Ecology and Evolution 2, 1843—1852.

Bennett, T.H., Flowers, T.J., Bromham, L., 2013. Repeated evolution of salt-tolerance in grasses. Biology Letters 9, 20130029.

Berg, J., Brandt, K.K., Al-Soud, W.A., Holm, P.E., Hansen, L.H., Sørensen, S.J., Nybroe, O., 2012. Selection for Cu-tolerant bacterial communities with altered composition, but unaltered richness, via long-term Cu exposure. Applied and Environmental Microbiology 78, 7438—7446.

Berga, M., 2013. Assembly Mechanisms in Aquatic Bacterial Communities: the Role of Disturbances, Dispersal and History (Ph.D. thesis). Uppsala University, Sweden.

Birch, H.F., 1958. The effect of soil drying on humus decomposition and nitrogen. Plant and Soil 10, 9—30.

Blagodatskaya, E., Kuzyakov, Y., 2013. Active microorganisms in soil: critical review of estimation criteria and approaches. Soil Biology & Biochemistry 67, 192—211.

Blagodatskaya, E.V., Anderson, T.-H., 1998. Interactive effects of pH and substrate quality on the fungal-to-bacterial ratio and qCO_2 of microbial communities in forest soils. Soil Biology & Biochemistry 30, 1269—1274.

Blagodatsky, S.A., Richter, O., 1998. Microbial growth in soil and nitrogen turnover: a theoretical model considering the activity state of microorganisms. Soil Biology & Biochemistry 30, 1743—1755.

Blanck, H., 2002. A critical review of procedures and approaches used for assessing pollution-induced community tolerance (PICT) in biotic communities. Human and Ecological Risk Assessment 8, 1003—1034.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37, 911–917.

Boivin, M.E.Y., Breure, A.M., Posthuma, L., Rutgers, M., 2002. Determination of field effects of contaminants – significance of pollution-induced community tolerance. Human and Ecological Risk Assessment 8, 1035–1055.

Bouvier, T.C., del Giorgio, P.A., 2002. Compositional changes in free-living bacterial communities along a salinity gradient in two temperate estuaries. Limnology and Oceanography 47, 453–470.

Bradford, M.A., 2013. Thermal adaptation of decomposer communities in warming soils. Frontiers in Microbiology 4 article 333.

Brandt, K.K., Sjøholm, O.R., Krogh, K.A., Halling-Sørensen, B., Nybroe, O., 2009. Increased pollution-induced bacterial community tolerance to sulfadiazine in soil hotspots amended with artificial root exudates. Environmental Science & Technology 43, 2963–2968.

Bronick, C.J., Lal, R., 2005. Soil structure and management: a review. Geoderma 124, 3–22.

Buringh, P., 1978. Food production potential of the world. In: Sinha, R. (Ed.), The World Food Problem: Consensus and Conflict. Pergamon Press, Oxford, UK, pp. 477–485.

Burns, R.G., DeForest, J.L., Marxsen, J., Sinsabaugh, R.L., Stromberger, M.E., Wallenstein, M.D., Weintraub, M.N., Zoppini, A., 2013. Soil enzymes in a changing environment: current knowledge and future directions. Soil Biology & Biochemistry 58, 216–234.

Bursy, J., Kuhlmann, A.U., Pittelkow, M., Hartmann, H., Jebbar, M., Pierik, A.J., Bremer, E., 2008. Synthesis and uptake of the compatible solutes ectoine and 5-hydroxyectoine by *Streptomyces coelicolor* A3(2) in response to salt and heat stresses. Applied and Environmental Microbiology 74, 7286–7296.

Campbell, B.J., Kirchman, D.L., 2013. Bacterial diversity, community structure and potential growth rates along an estuarine salinity gradient. The ISME Journal 7, 210–220.

Chandrajith, R., Chaturangani, D., Abeykoon, S., Barth, J.A.C., vsn Geldern, R., Edirisinghe, E.A.N.V., Dissanayake, C.B., 2014. Quantification of groundwater-seawater interaction in a coastal sandy aquifer system: a study from Panama, Sri Lanka. Environmental Earth Sciences 72, 867–877.

Chhabra, R., 1996. Soil Salinity and Water Quality. Balkema, Rotterdam, Netherlands.

Choudhary, O.P., Josan, A.S., Bajwa, M.S., Kapur, M.L., 2004. Effect of sustained sodic and saline-sodic irrigation and application of gypsum and farmyard manure on yield and quality of sugarcane under semi-arid conditions. Field Crops Research 87, 103–116.

Chowdhury, N., Marschner, P., Burns, R.G., 2011a. Response of microbial activity and community structure to decreasing soil osmotic and matric potential. Plant and Soil 344, 241–254.

Chowdhury, N., Marschner, P., Burns, R.G., 2011b. Soil microbial activity and community composition: impact of changes in matric and osmotic potential. Soil Biology & Biochemistry 43, 1229–1236.

Chowdhury, N., Yan, N., Islam, M.N., Marschner, P., 2011c. The extent of drying influences the flush of respiration after rewetting in non-saline and saline soils. Soil Biology & Biochemistry 43, 2265–2272.

Ciardi, C., Nannipieri, P., 1990. A comparison of methods for measuring ATP in soil. Soil Biology & Biochemistry 22, 725–727.

Clarholm, M., 1981. Protozoan grazing of bacteria in soil: impact and importance. Microbial Ecology 7, 343–350.

Clarholm, M., 1985. Interactions of bacteria, protozoa and plants leading to mineralization of soil-nitrogen. Soil Biology & Biochemistry 17, 181–187.

Clarke, C.J., George, R.J., Bell, R.W., Hatton, T.J., 2002. Dryland salinity in south-western Australia: its origins, remedies, and future research directions. Australian Journal of Soil Research 40, 93–113.

Cole, J.J., Findlay, S., Pace, M.L., 1988. Bacterial production in fresh and saltwater ecosystems – a cross-system overview. Marine Ecology Progress Series 43, 1–10.

Cotner, J.B., Ammerman, J.W., Peele, E.R., Bentzen, E., 1997. Phosphorus-limited bacterioplankton growth in the Sargasso Sea. Aquatic Microbial Ecology 13, 141–149.

Davidson, E.A., Janssens, I.A., 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature 440, 165–173.

De Boer, W., Folman, L.B., Summerbell, R.C., Boddy, L., 2005. Living in a fungal world: impact of fungi on soil bacterial niche development. FEMS Microbiology Reviews 29, 795–811.

De Graaff, M.A., Classen, A.T., Castro, H.F., Schadt, C.W., 2010. Labile soil carbon inputs mediate the soil microbial community composition and plant residue decomposition rates. New Phytologist 188, 1055–1064.

del Giorgio, P.A., Cole, J.J., 1998. Bacterial growth efficiency in natural aquatic systems. Annual Review of Ecology and Systematics 29, 503–541.

Díaz-Raviña, M., Bååth, E., 2001. Response of soil bacterial communities pre-exposed to different metals and reinoculated in an unpolluted soil. Soil Biology & Biochemistry 33, 241–248.

Djajakirana, G., Joergensen, R.G., Meyer, B., 1996. Ergosterol and microbial biomass relationship in soil. Biology and Fertility of Soils 22, 299–304.

Dumont, M.G., Murrell, J.C., 2005. Stable isotope probing – linking microbial identity to function. Nature Reviews Microbiology 3, 499–504.

Egamberdieva, D., Renella, G., Wirth, S., Islam, R., 2010. Secondary salinity effects on microbial biomass. Biology and Fertility of Soils 46, 445–449.

Elmajdoub, B., Marschner, P., 2013. Salinity reduces the ability of soil microbes to utilize cellulose. Biology and Fertility of Soils 49, 379–386.

Empadinhas, N., da Costa, M.S., 2008. Osmoadaptation mechanisms in pro-karyotes: distribution of compatible solutes. International Microbiology 11, 151–161.

Fernández-Calviño, D., Arias-Estevez, M., Díaz-Raviña, M., Bååth, E., 2011a. Bacterial pollution induced community tolerance (PICT) to Cu and interactions with pH in long-term polluted vineyard soils. Soil Biology & Biochemistry 43, 2324–2331.

Fernández-Calviño, D., Bååth, E., 2010. Growth response of the bacterial community to pH in soils differing in pH. FEMS Microbiology Ecology 73, 149–156.

Fernández-Calviño, D., Rousk, J., Brookes, P.C., Bååth, E., 2011b. Bacterial pH-optima for growth track soil pH, but are higher than expected at low pH. Soil Biology & Biochemistry 43, 1569–1575.

Fierer, N., Schimel, J.P., 2002. Effects of drying-rewetting frequency on soil carbon and nitrogen transformations. Soil Biology & Biochemistry 34, 777–787.

Fortunato, C.S., Herfort, L., Zuber, P., Baptista, A.M., Crump, B.C., 2012. Spatial variability overwhelms seasonal patterns in bacterioplankton communities across a river to ocean gradient. The ISME Journal 6, 554–563.

Frankenberger, W.T., Bingham, F.T., 1982. Influence of salinity on soil enzyme-activities. Soil Science Society of America Journal 46, 1173–1177.

Frostegård, A., Bååth, E., Tunlid, A., 1993. Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty-acid analysis. Soil Biology & Biochemistry 25, 723–730.

Garcia, C., Hernandez, T., 1996. Influence of salinity on the biological and biochemical activity of a calciorthird soil. Plant and Soil 178, 255–263.

Garcia-Pausas, J., Paterson, E., 2011. Microbial community abundance and structure are determinants of soil organic matter mineralisation in the presence of labile carbon. Soil Biology & Biochemistry 43, 1705–1713.

Gasol, J.M., Casamayor, E.O., Joint, I., Garde, K., Gustavson, K., Benlloch, S., Diez, B., Schauer, M., Massana, R., Pedrós-Alió, C., 2004. Control of heterotrophic pro-karyotic abundance and growth rate in hypersaline planktonic environments. Aquatic Microbial Ecology 34, 193–206.

German, D.P., Weintraub, M.N., Grandy, A.S., Lauber, C.L., Rinkes, Z.L., Allison, S.D., 2011. Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies. Soil Biology & Biochemistry 43, 1387–1397.

German, D.P., Marcelo, K.R.B., Stone, M.M., Allison, S.D., 2012. The Michaelis–Menten kinetics of soil extracellular enzymes in response to temperature: a cross-latitudinal study. Global Change Biology 18, 1468–1479.

Ghollarata, M., Raiesi, F., 2007. The adverse effects of soil salinization on the growth of *Trifolium alexandrinum* L. and associated microbial and biochemical properties in a soil from Iran. Soil Biology & Biochemistry 39, 1699–1702.

Ghoulam, C., Foursy, A., Fares, K., 2002. Effects of salt stress on growth, inorganic ions and proline accumulation in relation to osmotic adjustment in five sugar beet cultivars. Environmental and Experimental Botany 47, 39–50.

Göransson, H., Godbold, D.L., Jones, D.L., Rousk, J., 2013. Bacterial growth and respiration responses upon rewetting dry forest soils: impact of drought-legacy. Soil Biology & Biochemistry 57, 477–486.

Griffin, D.M., 1972. Ecology of Soil Fungi. Syracuse University Press, Syracuse.

Griffiths, B.S., Ritz, K., Bardgett, R.D., Cook, R., Christensen, S., Ekelund, F., Sørensen, S.J., Bååth, E., Bloem, J., de Ruiter, P.C., Dolfing, J., Nicolardot, B., 2000. Ecosystem response of pasture soil communities to fumigation-induced microbial diversity reductions: an examination of the biodiversity-ecosystem function relationship. Oikos 90, 279–294.

Griffiths, B.S., Ritz, K., Ebblewhite, N., Dobson, G., 1999. Soil microbial community structure: effects of substrate loading rates. Soil Biology & Biochemistry 31, 145–153.

Griffiths, B.S., Philippot, L., 2013. Insights into the resistance and resilience of the soil microbial community. FEMS Microbiology Reviews 37, 112–129.

Griggs, D., Stafford-Smith, M., Gaffney, O., Rockström, J., Öhman, M.C., Shyamsundar, P., Steffen, W., Glaser, G., Kanie, N., Noble, I., 2013. Policy: sustainable development goals for people and planet. Nature 495, 305–307.

Hamdan, L.J., Coffin, R.B., Sikaroodi, M., Greinert, J., Treude, T., Gillevet, P.M., 2013. Ocean currents shape the microbiome of the Arctic marine sediment. ISME Journal 7, 685–696.

Haque, S.A., 2006. Salinity problems and crop production in coastal regions of Bangladesh. Pakistan Journal of Botany 38, 1359–1365.

Hart, S.C., Nason, G.E., Myrold, D.D., Perry, D.A., 1994. Dynamics of gross nitrogen transformations in an old-growth forest: the carbon connection. Ecology 75, 880–891.

Hartley, I.P., Hopkins, D.W., Garnett, M.H., Sommerkorn, M., Wookey, P.A., 2008. Soil microbial respiration in arctic soil does not acclimate to temperature. Ecology Letters 11, 1092–1100.

Hobbie, J.E., Hobbie, E.A., 2013. Microbes in nature are limited by carbon and energy: the starving-survival lifestyle in soil and consequences for estimating microbial rates. Frontiers in Microbiology 4 article 324.

Horner-Devine, M.C., Carney, K.M., Bohannan, B.J.M., 2004. An ecological perspective on bacterial biodiversity. Proceedings of the Royal Society B-Biological Sciences 271, 113–122.

Hosono, K., 1992. Effect of salt stress on lipid-composition and membrane fluidity of the salt-tolerant yeast Zygosaccharomyces-Rouxii. Journal of General Microbiology 138, 91–96.

Iwai, C.B., Oo, A.N., Topark-ngarm, B., 2012. Soil property and microbial activity in natural salt affected soils in an alternating wet-dry tropical climate. Gedoerma 189, 144–152.

Jackson, R.B., Jobbagy, E.G., Avissar, R., Roy, S.B., Barrett, D.J., Cook, C.W., Farley, K.A., le Maitre, D.C., McCarl, B.A., Murray, B.C., 2005. Trading water for carbon with biological sequestration. Science 310, 1944–1947.

Jenkinson, D.S., Ladd, J.N., 1981. Microbial biomass in soil: measurement and turnover. In: Paul, E.A., Ladd, J.N. (Eds.), Soil Biochemistry, vol. 5. Marcel Dekker, New York, pp. 415–471.

Jobbagy, E.G., Jackson, R.B., 2004. Groundwater use and salinization with grassland afforestation. Global Change Biology 10, 1299–1312.

Joergensen, R.G., Wichern, F., 2008. Quantitative assessment of the fungal contribution to microbial tissue in soil. Soil Biology & Biochemistry 40, 2977–2991.

Joergensen, R.G., Wu, J.S., Brookes, P.C., 2011. Measuring soil microbial biomass using an automated procedure. Soil Biology & Biochemistry 43, 873–876.

Johnson, D.D., Guenzi, W.D., 1963. Influence of salts on ammonium oxidation and carbon dioxide evolution from soil. Soil Science Society of America Proceedings 27, 663–666.

Kashian, D.R., Zuellig, R.E., Mitchell, K.A., Clements, W.H., 2007. The cost of tolerance: sensitivity of stream benthic communities to UV-B and metals. Ecological Applications 17, 365–375.

Kakumanu, M.L., Cantrell, C.L., Williams, M.A., 2013. Microbial community response to varying magnitudes of desiccation in soil: a test of the osmolyte accumulation hypothesis. Soil Biology & Biochemistry 57, 644–653.

Kakumanu, M.L., Williams, M.A., 2014. Osmolyte dynamics and microbial communities vary in response to osmotic more than matric water deficit gradients in two soils. Soil Biology & Biochemistry 79, 14–24.

Katerji, N., van Hoorn, J.W., Hamdy, A., Mastrorilli, M., 2003. Salinity effect on crop development and yield, analysis of salt tolerance according to several classification methods. Agricultural Water Management 62, 37–66.

Keiser, A.D., Strickland, M.S., Fierer, N., Bradford, M.A., 2011. The effect of resource history on the functioning of soil microbial communities is maintained across time. Biogeosciences 8, 1477–1486.

Kempf, B., Bremer, E., 1998. Uptake and synthesis of compatible solutes as microbial stress responses to high-osmolality environments. Archives of Microbiology 170, 319–330.

Killham, K., Firestone, M.K., 1984. Salt stress control of intracellular solutes in streptomycetes indigenous to saline soils. Applied and Environmental Microbiology 47, 301–306.

Killham, K., 1994. Soil Ecology. Cambridge University Press, Cambridge.

Kinraide, T.B., 1999. Interactions among Ca^{2+}, Na^+ and K^+ in salinity toxicity: quantitative resolution of multiple toxic and ameliorative effects. Journal of Experimental Botany 50, 1495–1505.

Kirchman, D., 2001. Measuring bacterial biomass production and growth rates from leucine incorporation in natural aquatic environments. Methods in Microbiology 30, 227–237.

Kirchman, D.L., 2012. Processes in Microbial Ecology. Oxford University Press, Oxford; New York.

Kirschbaum, M.U.F., 1995. The temperature-dependence of soil organic-matter decomposition, and the effect of global warming on soil organic-C storage. Soil Biology & Biochemistry 27, 753–760.

Kleber, M., 2010. What is recalcitrant soil organic matter? Environmental Chemistry 7, 320–332.

Kögel-Knabner, I., Ekschmitt, K., Flessa, H., Guggenberger, G., Matzner, E., Marschner, B., von Luetzow, M., 2008. An integrative approach of organic matter stabilization in temperate soils: linking chemistry, physics, and biology. Journal of Plant Nutrition and Soil Science 171, 5–13.

Ladeiro, B., 2012. Saline agriculture in the 21st century: using salt contaminated resources to cope food requirements. Journal of Botany 2012. Article ID 310705.

Langenheder, S., Lindström, E.S., Tranvik, L.J., 2006. Structure and function of bacterial communities emerging from different sources under identical conditions. Applied and Environmental Microbiology 72, 212–220.

Langenheder, S., Ragnarsson, H., 2007. The role of environmental and spatial factors for the composition of aquatic bacterial communities. Ecology 88, 2154–2161.

Laura, R.D., 1974. Effects of neutral salts on carbon and nitrogen mineralization of organic-matter in soil. Plant and Soil 41, 113–127.

Lindström, E.S., Östman, Ö., 2011. The importance of dispersal for bacterial community composition and functioning. PLOS One 6, e25883.

Logares, R., Lindström, E.S., Langenheder, S., Logue, J.B., Paterson, H., Laybourn-Parry, J., Rengefors, K., Tranvik, L., Bertilsson, S., 2013. Biogeography of bacterial communities exposed to progressive long-term environmental change. The ISME Journal 7, 937–948.

Lozupone, C.A., Knight, R., 2007. Global patterns in bacterial diversity. Proceedings of the National Academy of Sciences of the United States of America 104, 11436–11440.

Malik, A., Gleixner, G., 2013. Importance of microbial soil organic matter processing in dissolved organic carbon production. FEMS Microbiology Ecology 86, 139–148.

Mamilov, A.S., Dilly, O.A., 2002. Soil microbial eco-physiology as affected by short-term variations in environmental conditions. Soil Biology & Biochemistry 34, 1283–1290.

Manzoni, S., Schimel, J.P., Porporato, A., 2012. Responses of soil microbial communities to water stress: results from a metaanalsysis. Ecology 93, 930–938.

Marschner, B., Brodowski, S., Dreves, A., Gleixner, G., Gude, A., Grootes, P.M., Hamer, U., Heim, A., Jandl, G., Ji, R., Kaiser, K., Kalbitz, K., Kramer, C., Leinweber, P., Rethemeyer, J., Schaeffer, A., Schmidt, M.W.I., Schwark, L., Wiesenberg, G.L.B., 2008. How relevant is recalcitrance for the stabilization of

organic matter in soils? Journal of Plant Nutrition and Soil Science-Zeitschrift Fur Pflanzenernahrung Und Bodenkunde 171, 91–110.

Marschner, H., 1995. Mineral Nutrition of Higher Plants. Academic Press, London.

Martinez-Beltran, J., Manzur, K.L., 2005. Overview of Salinity Problems in the World and FAO Strategies to Address the Problem. Pages 311–314 in International Salinity Forum-managing Saline Soils and Water: Science, Technology and Social Issues. USDA-ARS Salinity Laboratory, Riverside, California, USA.

Mavi, M.S., Marschner, P., 2012. Drying and wetting in saline and saline-sodic soils-effects on microbial activity, biomass and dissolved organic carbon. Plant and Soil 355, 51–62.

Mavi, M.S., Marschner, P., 2013. Salinity affects the response of soil microbial activity and biomass to addition of carbon and nitrogen. Soil Research 51, 68–75.

Mavi, M.S., Sanderman, J., Chittleborough, D.J., Cox, J.W., Marschner, P., 2012. Sorption of dissolved organic matter in salt-affected soils: effect of salinity, sodicity and texture. Science of the Total Environment 435, 337–344.

Meloni, D., Oliva, M., Ruiz, H., Martinez, C., 2001. Contribution of proline and inorganic solutes to osmotic adjustment in cotton under salt stress. Journal of Plant Nutrition 24, 599–612.

Mikha, M.M., Rice, C.W., Milliken, G.A., 2005. Carbon and nitrogen mineralization as affected by drying and wetting cycles. Soil Biology & Biochemistry 37, 339–347.

Mikutta, R., Mikutta, C., Kalbitz, K., Scheel, T., Kaiser, K., Jahn, R., 2007. Biodegradation of forest floor organic matter bound to minerals via different binding mechanisms. Geochimica Et Cosmochimica Acta 71, 2569–2590.

Mille-Lindblom, C., von Wachenfeldt, E., Tranvik, L.J., 2004. Ergosterol as a measure of living fungal biomass: persistence in environmental samples after fungal death. Journal of Microbiological Methods 59, 253–262.

Miltner, A., Bombach, P., Schmidt-Brucken, B., Kastner, M., 2012. SOM genesis: microbial biomass as a significant source. Biogeochemistry 111, 41–55.

Mohamed, D.J., Martiny, J.B.H., 2011. Patterns of fungal diversity and composition along a salinity gradient. The ISME Journal 5, 379–388.

Monod, J., 1949. The growth of bacterial cultures. Annual Review of Microbiology 3, 371–394.

Muhammad, S., Müller, T., Joergensen, R.G., 2006. Decomposition of pea and maize straw in Pakistani soils along a gradient in salinity. Biology and Fertility of Soils 43, 93–101.

Muhammad, S., Müller, T., Joergensen, R.G., 2008. Relationships between soil biological and other soil properties in saline and alkaline arable soils from the Pakistani Punjab. Journal of Arid Environments 72, 448–457.

Nannipieri, P., 2006. Role of stabilised enzymes in microbial ecology and enzyme extraction from soil with potential applications in soil proteomics. In: Nannipieri, P., Smalla, K. (Eds.), Nucleic Acids and Proteins in Soil. Springer, Berlin, Germany.

Nelson, P.N., Ladd, J.N., Oades, J.M., 1996. Decomposition of ^{14}C-labelled plant material in a salt-affected soil. Soil Biology & Biochemistry 28, 433–441.

Oades, J.M., 1984. Soil organic-matter and structural stability – mechanisms and implications for management. Plant and Soil 76, 319–337.

Oades, J.M., 1988. The retention of organic-matter in soils. Biogeochemistry 5, 35–70.

Oren, A., 1999. Bioenergetic aspects of halophilism. Microbiology and Molecular Biology Reviews 63, 334–348.

Oren, A., 2008. Microbial life at high salt concentrations: phylogenetic and metabolic diversity. Saline Systems 4 article 2.

Pal, B., Singh, C., Singh, H., 1984. Barley yield under saline water cultivation. Plant and Soil 81, 221–228.

Pankhurst, C.E., Yu, S., Hawke, B.G., Harch, B.D., 2001. Capacity of fatty acid profiles and substrate utilization patterns to describe differences in soil microbial communities associated with increased salinity or alkalinity at three locations in South Australia. Biology and Fertility of Soils 33, 204–217.

Parida, A.K., Das, A.B., 2005. Salt tolerance and salinity effects on plants: a review. Ecotoxicology and Environmental Safety 60, 324–349.

Pathak, H., Rao, D.L.N., 1998. Carbon and nitrogen mineralization from added organic matter in saline and alkali soils. Soil Biology & Biochemistry 30, 695–702.

Pedrós-Alió, C., Calderón-Paz, J.I., MacLean, M.H., Medina, G., Marrasé, C., Gasol, J.M., Guixa-Boixereu, N., 2000. The microbial food web along salinity gradients. FEMS Microbiology Ecology 32, 143–155.

Pettersson, M., Bååth, E., 2013. Importance of inoculum properties on the structure and growth of bacterial communities during recolonisation of humus soil with different pH. Microbial Ecology 66, 416–426.

Philippot, L., Spor, A., Henault, C., Bru, D., Bizouard, F., Jones, C.M., Sarr, A., Maron, P.A., 2013. Loss in microbial diversity affects nitrogen cycling in soil. The ISME Journal 7, 1609–1619.

Placella, S.A., Brodie, E.L., Firestone, M.K., 2012. Rainfall-induced carbon dioxide pulses from sequential resuscitation of phylogenetically clustered microbial groups. Proceedings of the National Academy of Sciences of the USA 109, 10931–10936.

Raich, J.W., Potter, C.S., 1995. Global patterns of carbon-dioxide emissions from soils. Global Biogeochemical Cycles 9, 23–36.

Ramírez-Fuentes, E., Luna-Guido, M.L., Ponce-Mendova, A., Van den Broeck, E., Dendooven, L., 2002. Incorporation of glucose-^{14}C and NH_4^+ in microbial biomass of alkaline saline soil. Biology and Fertility of Soils 36, 269–275.

Reed, H.E., Martiny, J.B.H., 2013. Microbial composition affects the functioning of estuarine sediments. The ISME Journal 7, 868–879.

Reischke, S., Rousk, J., Bååth, E., 2014. The effects of glucose loading rates on bacterial and fungal growth in soil. Soil Biology and Biochemistry 70, 88–95.

Rengasamy, P., 2006a. Soil salinity and sodicity. In: Stevens, D. (Ed.), Growing Crops with Reclaimed Wastewater. CSIRO, Victoria, pp. 125–138.

Rengasamy, P., 2006b. World salinization with emphasis on Australia. Journal of Experimental Botany 57, 1017–1023.

Richards, R., Dennett, C., Qualset, C., Epstein, E., Norlyn, J., Winslow, M., 1987. Variation in yield of grain and biomass in wheat, barley, and triticale in a salt-affected field. Field and Crops Research 15, 277–287.

Richards, R., 1983. Should selection for yield in saline regions be made on saline or non-saline soils? Euphytica 32, 431–438.

Rietz, D.N., Haynes, R.J., 2003. Effects of irrigation-induced salinity and sodicity on soil microbial activity. Soil Biology & Biochemistry 35, 845–854.

Rousk, J., Aldén Demoling, L., Bååth, E., 2009. Contrasting short-term antibiotic effects on respiration and bacterial growth compromises the validity of the selective respiratory inhibition technique to distinguish fungi and bacteria. Microbial Ecology 58, 75–85.

Rousk, J., Brookes, P.C., Bååth, E., 2010. Investigating the mechanisms for the opposing pH relationships of fungal and bacterial growth in soil. Soil Biology & Biochemistry 42, 926–934.

Rousk, J., Bååth, E., 2011. Growth of saprotrophic fungi and bacteria in soil. FEMS Microbiology Ecology 78, 17–30.

Rousk, J., Brookes, P.C., Glanville, H.C., Jones, D.L., 2011a. Lack of correlation between turnover of low-molecular-weight dissolved organic carbon and differences in microbial community composition or growth across a soil pH gradient. Applied and Environmental Microbiology 77, 2791–2795.

Rousk, J., Elyaagubi, F.K., Jones, D.L., Godbold, D.L., 2011b. Bacterial salt tolerance is unrelated to soil salinity across an arid agroecosystem salinity gradient. Soil Biology & Biochemistry 43, 1881–1887.

Rousk, J., Frey, S.D., Bååth, E., 2012. Temperature adaptation of bacterial communities in experimentally warmed forest soils. Global Change Biology 18, 3252–3258.

Rousk, J., Smith, A.R., Jones, D.L., 2013. Investigating the long-term legacy of drought and warming on the soil microbial communities across five European shrubland ecosystems. Global Change Biology 19, 3872–3884.

Rustad, L.E., Huntington, T.G., Boone, R.D., 2000. Controls on soil respiration: implications for climate change. Biogeochemistry 48, 1–6.

Ruzicka, S., Edgerton, D., Norman, M., Hill, T., 2000. The utility of ergosterol as a bioindicator of fungi in temperate soils. Soil Biology & Biochemistry 32, 989–1005.

Sardinha, M., Müller, T., Schmeisky, H., Joergensen, R.G., 2003. Microbial performance in soils along a salinity gradient under acidic conditions. Applied Soil Ecology 23, 237–244.

Saviozzi, A., Cardelli, R., Di Puccio, R., 2011. Impact of salinity on soil biological activities: a laboratory experiment. Communications in Soil Science and Plant Analysis 42, 358–367.

Schimel, J., Balser, T.C., Wallenstein, M., 2007. Microbial stress-response physiology and its implications for ecosystem function. Ecology 88, 1386–1394.

Schimel, J.P., Gulledge, J.M., Clein-Curley, J.S., Lindstrom, J.E., Braddock, J.F., 1999. Moisture effects on microbial activity and community structure in decomposing birch litter in the Alaskan taiga. Soil Biology & Biochemistry 31, 831–838.

Schimel, J.P., Weintraub, M.N., 2003. The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. Soil Biology & Biochemistry 35, 549–563.

Schlesinger, W.H., 1997. Biogeochemistry: an Analysis of Global Change, second ed. Academic Press, San Diego, Calif.

Schmidt, M.W.I., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I.A., Kleber, M., Kögel-Knabner, I., Lehmann, J., Manning, D.A.C., Nannipieri, P., Rasse, D.P., Weiner, S., Trumbore, S.E., 2011. Persistence of soil organic matter as an ecosystem property. Nature 478, 49–56.

Schultz, G.E., White, E.D., Ducklow, H.W., 2003. Bacterioplankton dynamics in the York River estuary: primary influence of temperature and freshwater inputs. Aquatic Microbial Ecology 30, 135–148.

Setia, R., Marschner, P., 2013. Carbon mineralization in saline soils as affected by residue composition and water potential. Biology and Fertility of Soils 49, 71–77.

Setia, R., Marschner, P., Baldock, J., Chittleborough, D., 2010. Is CO_2 evolution in saline soils affected by an osmotic effect and calcium carbonate? Biology and Fertility of Soils 46, 781–792.

Setia, R., Marschner, P., Baldock, J., Chittleborough, D., Smith, P., Smith, J., 2011a. Salinity effects on carbon mineralization in soils of varying texture. Soil Biology & Biochemistry 43, 1908–1916.

Setia, R., Marschner, P., Baldock, J., Chittleborough, D., Verma, V., 2011b. Relationships between carbon dioxide emission and soil properties in salt-affected landscapes. Soil Biology & Biochemistry 43, 667–674.

Setia, R., Rengasamy, P., Marschner, P., 2013. Effect of exchangeable cation concentration on sorption and desorption of dissolved organic carbon in saline soils. Science of the Total Environment 465, 226–232.

Setia, R., Smith, P., Marschner, P., Gottschalk, P., Baldock, J., Verma, V., Setia, D., Smith, J., 2012. Simulation of salinity effects on past, present, and future soil organic carbon stocks. Environmental Science & Technology 46, 1624–1631.

Severin, I., Östman, Ö., Lindström, E.S., 2013. Variable effects of dispersal on productivity of bacterial communities due to changes in functional trait composition. PLOS One 8 article e80825.

Shade, A., Peter, H., Allison, S.D., Baho, D.L., Berga, M., Burgmann, H., Huber, D.H., Langenheder, S., Lennon, J.T., Martiny, J.B.H., Matulich, K.L., Schmidt, T.M.,

Handelsman, J., 2012. Fundamentals of microbial community resistance and resilience. Frontiers in Microbiology 3 article 417.

Shainberg, I., Letey, J., 1984. Response of soils to sodic and saline conditions. Hilgardia 52, 1–57.

Six, J., Frey, S.D., Thiet, R.K., Batten, K.M., 2006. Bacterial and fungal contributions to carbon sequestration in agroecosystems. Soil Science Society of America Journal 70, 555–569.

Six, J., Paustian, K., Elliott, E.T., Combrink, C., 2000. Soil structure and organic matter: I. Distribution of aggregate-size classes and aggregate-associated carbon. Soil Science Society of America Journal 64, 681–689.

Sjösted, J., Koch-Schmidt, P., Pontarp, M., Canbäck, B., Tunlid, A., Lundberg, P., Hagström, Å., Riemann, L., 2012. Recruitment of members from the rare biosphere of marine bacterioplankton communities after an environmental disturbance. Applied and Environmental Microbiology 78, 1361–1369.

Soomro, A., Mirjat, M., Oad, F., Soomro, H., Samo, M., Oad, N., 2001. Effect of irrigation intervals on soil salinity and cotton yield. OnLine Journal of Biological Sciences 1, 472–474.

Stefanowicz, A.M., Niklinska, M., Laskowski, R., 2009. Pollution-induced tolerance of soil bacterial communities in meadow and forest ecosystems polluted with heavy metals. European Journal of Soil Biology 45, 363–369.

Stevens, R.M., Partington, D.L., 2013. Grapevine recovery from saline irrigation was incomplete after four seasons of non-saline irrigation. Agricultural Water Management 122, 39–45.

Strickland, M.S., Rousk, J., 2010. Considering fungal:bacterial dominance in soils — methods, controls, and ecosystem implications. Soil Biology & Biochemistry 42, 1385–1395.

Suberkropp, K., 2001. Fungal growth, production, and sporulation during leaf decomposition in two streams. Applied and Environmental Microbiology 67, 5063–5068.

Sumner, M.E., Naida, R., 1998. Sodic Soils: Distribution, Properties, Management, and Environmental Consequences. Oxford University Press, New York.

Szabolcs, I., 1989. Salt-affected Soils. CRC Press, Boca Raton, Fla.

Teira, E., Martinez-Garcia, S., Lonborg, C., Alvarez-Salgado, X.A., 2011. Betaproteobacteria growth and nitrification rates during long-term natural dissolved organic matter decomposition experiments. Aquatic Microbial Ecology 63, 19–27.

Torsvik, V., Ovreas, L., 2002. Microbial diversity and function in soil: from genes to ecosystems. Current Opinion in Microbiology 5, 240–245.

Trabelsi, D., Mengoni, A., Aouani, M.E., Bazzicalupo, M., Mhamdi, R., 2010. Genetic diversity and salt tolerance of Sinorhizobium populations from two Tunisian soils. Annals of Microbiology 60, 541–547.

Tripathi, S., Kumari, S., Chakraborty, A., Gupta, A., Chakrabarti, K., Bandyapadhyay, B.K., 2006. Microbial biomass and its activities in salt-affected coastal soils. Biology and Fertility of Soils 42, 273–277.

Troussellier, M., Schafer, H., Batailler, N., Bernard, L., Courties, C., Lebaron, P., Muyzer, G., Servais, P., Vives-Rego, J., 2002. Bacterial activity and genetic richness along an estuarine gradient (Rhone River plume, France). Aquatic Microbial Ecology 28, 13–24.

UNEP, 2007. Global Environment Outlook 4: Environment for Development. United Nations Environment Programme. Progress Press, Valetta, Malta.

US Salinity Laboratory Staff, 1954. Diagnosis and Improvement of Saline and Alkali Soils. In: USDA Handbook No. 60. US Government Printing Office, Washington D.C.

Velvis, H., 1997. Evaluation of the selective respiratory inhibition method for measuring the ratio of fungal:bacterial activity in acid agricultural soils. Biology and Fertility of Soils 25, 354–360.

Ventosa, A., Nieto, J.J., Oren, A., 1998. Biology of moderately halophilic aerobic bacteria. Microbiology and Molecular Biology Reviews 62, 504–544.

Waksman, S.A., Gerretsen, F.C., 1931. Influence of temperature and moisture upon the nature and extent of decomposition of plant residues by microorganisms. Ecology 12, 33–60.

Walsh, D.A., Papke, R.T., Doolittle, W.F., 2005. Archaeal diversity along a soil salinity gradient prone to disturbance. Environmental Microbiology 7, 1655–1666.

Wang, Y., Sheng, H.F., He, Y., Wu, J.Y., Jiang, Y.X., Tam, N.F.Y., Zhou, H.W., 2012. Comparison of the levels of bacterial diversity in freshwater, intertidal wetland, and marine sediments by using millions of Illumina tags. Applied and Environmental Microbiology 78, 8264–8271.

Wardle, D.A., 1992. A comparative assessment of factors which influence microbial biomass carbon and nitrogen levels in soil. Biological Reviews 67, 321–358.

Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setälä, H., van der Putten, W.H., Wall, D.H., 2004. Ecological linkages between aboveground and belowground biota. Science 304, 1629–1633.

Wardle, D.A., Ghani, A., 1995. A critique of the microbial metabolic quotient (qCO_2) as a bioindicator of disturbance and ecosystem development. Soil Biology & Biochemistry 27, 1601–1610.

Warren, C.R., 2014. Response of osmolytes in soil to drying and rewetting. Soil Biology and Biochemistry 70, 22–32.

Wichern, F., Lobe, I., Amelung, W., Müller, T., Joergensen, R.G., Buerkert, A., 2004. Changes in amino acid enantiomers and microbial performance in soils from a subtropical mountain oasis in Oman abandoned for different periods. Biology and Fertility of Soils 39, 398–406.

Wichern, J., Wichern, F., Joergensen, R.G., 2006. Impact of salinity on soil microbial communities and the decomposition of maize in acidic soils. Geoderma 137, 100–108.

Williams, M.A., Xia, K., 2009. Characterization of the water soluble soil organic pool following the rewetting of dry soil in a drought-prone tallgrass prairie. Soil Biology & Biochemistry 41, 21–28.

Wong, V.N.L., Dalal, R.C., Greene, R.S.B., 2008. Salinity and sodicity effects on respiration and microbial biomass of soil. Biology and Fertility of Soils 44, 943–953.

Wong, V.N.L., Dalal, R.C., Greene, R.S.B., 2009. Carbon dynamics of sodic and saline soils following gypsum and organic material additions: a laboratory incubation. Applied Soil Ecology 41, 29–40.

Wong, V.N.L., Greene, R.S.B., Dalal, R.C., Murphy, B.W., 2010. Soil carbon dynamics in saline and sodic soils: a review. Soil Use and Management 26, 2–11.

Yan, N., Marschner, P., 2012. Response of microbial activity and biomass to increasing salinity depends on the final salinity, not the original salinity. Soil Biology & Biochemistry 53, 50–55.

Yan, N., Marschner, P., 2013a. Microbial activity and biomass recover rapidly after leaching of saline soils. Biology and Fertility of Soils 49, 367–371.

Yan, N., Marschner, P., 2013b. Response of soil respiration and microbial biomass to changing EC in saline soils. Soil Biology & Biochemistry 65, 322–328.

Yuan, B.C., Li, Z.Z., Liu, H., Gao, M., Zhang, Y.Y., 2007. Microbial biomass and activity in salt affected soils under arid conditions. Applied Soil Ecology 35, 319–328.

Zabaloy, M.C., Garland, J.L., Gomez, M.A., 2010. Assessment of the impact of 2,4-dichlorophenoxyacetic acid (2,4-D) on indigenous herbicide-degrading bacteria and microbial community function in an agricultural soil. Applied Soil Ecology 46, 240–246.

Zhao, X.R., Brookes, P.C., 2005. Does soil ergosterol concentration provide a reliable estimate of soil fungal biomass? Soil Biology & Biochemistry 37, 311–317.

Zhu, J.K., 2001. Plant salt tolerance. Trends in Plant Science 6, 66–71.

Comparative Toxicities of Salts on Microbial Processes in Soil

Kristin M. Rath,[a,b] Arpita Maheshwari,[a] Per Bengtson,[a] Johannes Rousk[a]

Section of Microbial Ecology, Department of Biology, Lund University, Lund, Sweden[a]; Centre for Environmental and Climate Research (CEC), Lund University, Lund, Sweden[b]

Soil salinization is a growing threat to global agriculture and carbon sequestration, but to date it remains unclear how microbial processes will respond. We studied the acute response to salt exposure of a range of anabolic and catabolic microbial processes, including bacterial (leucine incorporation) and fungal (acetate incorporation into ergosterol) growth rates, respiration, and gross N mineralization and nitrification rates. To distinguish effects of specific ions from those of overall ionic strength, we compared the addition of four salts frequently associated with soil salinization ($NaCl$, KCl, Na_2SO_4, and K_2SO_4) to a nonsaline soil. To compare the tolerance of different microbial processes to salt and to interrelate the toxicity of different salts, concentration-response relationships were established. Growth-based measurements revealed that fungi were more resistant to salt exposure than bacteria. Effects by salt on C and N mineralization were indistinguishable, and in contrast to previous studies, nitrification was not found to be more sensitive to salt exposure than other microbial processes. The ion-specific toxicity of certain salts could be observed only for respiration, which was less inhibited by salts containing SO_4^{2-} than Cl^- salts, in contrast to the microbial growth assessments. This suggested that the inhibition of microbial growth was explained solely by total ionic strength, while ion-specific toxicity also should be considered for effects on microbial decomposition. This difference resulted in an apparent reduction of microbial growth efficiency in response to exposure to SO_4^{2-} salts but not to Cl^- salts; no evidence was found to distinguish K^+ and Na^+ salts.

Soil salinization affects a large area of land globally and has become a major threat to agricultural productivity and food security (1). Due to the wide distribution of salt-affected soils around the world (2, 3), it is important to understand the influence of salinity on the soil microbial community. The soil microbial decomposer community plays an essential role in the decomposition and stabilization of soil organic matter (SOM), as well as the cycling of nutrients vital for plant growth. How substrate during decomposition is allocated to either microbial biomass production or respiration determines the microbial growth efficiency (MGE), which is an important parameter for the C sequestration potential of a soil (4). The potential for soil C storage could be compromised by disturbances or unfavorable environmental conditions that reduce microbial growth efficiencies due to the metabolic burden they place on microbial cells (5).

It is generally held that fungus-dominated communities have a higher MGE than communities dominated by bacteria (4). Thus, changes in the relative contribution of bacteria and fungi to the soil microbial community are thought to reflect changes in ecosystem processes, such as decomposition, C sequestration potential, and nutrient cycling (6, 7). It is unclear whether fungi and bacteria are affected by salt exposure to a similar degree or if there are differences in salt sensitivity between these two major decomposer groups. It has been shown that fungi are more resistant to osmotic pressure, illustrated by their higher tolerance to high concentrations of low-molecular-weight organic compounds (8, 9). In addition, fungi also have been found to be more resistant to low water potentials brought about by decreasing soil moisture than most bacteria (10, 11). In soils exposed to salinity, both higher (12, 13) and lower (14–17) levels of contribution of fungi to the microbial community have been observed.

Often the influence of soil salinity on the soil microbial community has been studied using total microbial biomass measurements. However, the connection between the total microbial biomass and microbial contribution to soil processes is tenuous at best (6, 18, 19), rendering biomass a poor predictor for process rates carried out by the microbial community. Instead, responses in processes carried out by the active and growing part of the microbial community can be employed to detect inhibition by exposure to salts. For instance, salt additions have been found to influence and reduce microbial activity, measured as respiration (12, 20–23) or N transformation rates (22, 24). To date, there is a lack of comparative studies on the degree of sensitivity of a comprehensive range of different microbial processes. Processes showing differential sensitivity to salinity could have implications for soil biogeochemical cycles and the ecology of microorganisms, as well as the identification of informative endpoints for toxicity assessments. In addition, not all salts associated with soil salinization have the same effect on the microbial community. Differences in toxicity have been found between, e.g., SO_4^{2-} and Cl^- salts (25–30) as well as K^+ and Na^+ salts (28). However, few studies have been designed to explicitly compare the toxicity of different salts using a range of processes.

The aim of this study was to conduct a comparative analysis of

the sensitivity of a range of different microbial processes to short-term salt exposure in a nonsaline soil. In the first part of the study, soil was exposed to a range of NaCl concentrations. The acute growth responses of bacteria and fungi were compared to assess differences in their tolerance to salinity. In addition, growth processes were compared to catabolic processes, including C and N mineralization and nitrification, to investigate the potential for salts to induce a shift in SOM dynamics and nutrient cycling. Considering the predicted higher tolerance of fungi to osmotic pressure, we hypothesized that fungal growth would show a higher tolerance to salts associated with soil salinization than bacterial growth. Further, we predicted that, as a symptom of the cost of physiological measures to cope with high osmotic potentials, microorganisms allocate substrate away from biomass production toward maintenance functions, leading to a situation where catabolic processes would be less inhibited by salt exposure than anabolic or growth-related processes. Incubation times were kept short to ensure that the measured responses are direct responses to salt exposure rather than inhibition confounded by the recovery due to a shift toward a more tolerant community. In the second part of the study, we conducted a comparative assessment of the toxicity of salts common in saline soils ($NaCl$, KCl, K_2SO_4, and Na_2SO_4) on respiration as well as fungal and bacterial growth. We hypothesized that Cl^- salts would be more toxic than SO_4^{2-} salts, and that Na^+ salts would be more toxic than K^+ salts. We also predicted that irrespective of the type of salt used, fungi would be more resistant than bacteria and respiration less inhibited than growth.

MATERIALS AND METHODS

Soil sampling and characterization. Soil was collected from a grassland site situated in Vomb, southern Sweden (55°40′27″N, 13°32′45″E). The soil is a well-drained sandy grassland soil. Multiple soil samples were collected with a spade from pits dug to a depth of ca. 20 cm and combined into composite samples, homogenized, and sieved (<2.8 mm). Samples were collected at several time points from the same site: September 2013, October 2013, November 2014, and December 2014.

Following sieving and homogenization, the water content of the soil was determined gravimetrically (105°C for 24 h), and the organic matter (OM) content was measured as loss on ignition (600°C for 10 h). Electrical conductivity (EC) and pH measurements were conducted in a 1:5 soil-water suspension. To measure NH_4^+ and NO_3^- concentrations, diffusion traps were placed in a KCl soil extract. The total microbial biomass was determined using substrate-induced respiration (SIR) (31) by adding 6 mg of glucose per g soil. After 2 h of incubation, CO_2 was measured using a gas chromatograph (GC) equipped with a methanizer and a flame ionization detector. The measured respiration rate was converted to microbial biomass C using the relationship that 1 mg CO_2 h^{-1} corresponds to 20 mg biomass-C (31). These SIR estimates of microbial biomass matched previous assessments of biomass from the same soils (data not shown).

Fungal biomass C was estimated by extracting and quantifying the amount of ergosterol in the soil sample. We assumed a fungal ergosterol content of 5 mg g^{-1} fungal biomass and a fungal C content of 45% (32, 33). Bacterial biomass C was estimated using a mass balance of fungal biomass C to total microbial biomass C.

Soil samples subsequently were stored in gas-permeable polyethylene bags at 4°C until further analysis. Before the start of the experiment, soil was kept at 22°C for 2 days. The study was divided into two parts, referred to as experiments 1 and 2 below.

Experiment 1. In the first part of the study, the sensitivities of bacterial and fungal growth, respiration, and gross N mineralization and nitrification rates to sodium chloride (NaCl) were determined. Bacterial growth measurements were repeated in samples from all four sampling time

points, fungal growth and respiration were measured in samples collected in September 2013 and December 2014, and gross N mineralization and nitrification rates were measured in the sample collected in October 2013. Before measuring microbial growth and process rates, NaCl was added to the soils in 8 to 12 different concentrations, which covered a range from 0 to 3,600 μmol NaCl per g soil in a series of 3-fold dilution steps, together with 100 μl distilled water per g soil. The salt additions resulted in a range of $EC_{1:5}$ values from 0.06 to 65.6 dS m^{-1} (i.e., ca. 0.6 to 700 dS m^{-1} in a saturated soil paste [EC_e] [21]). Generally, a soil is described as saline if the EC_e is higher than 4 dS m^{-1}, but studies of saline soils frequently include soils with a more than 10-fold higher EC_e (23). Following the addition of NaCl, soils were incubated at room temperature for 1.5 h before microbial variables were determined.

Experiment 2. In the second part of the study, we compared the toxic effects of salts common in saline soils, namely, NaCl, potassium chloride (KCl), potassium sulfate (K_2SO_4), and sodium sulfate (Na_2SO_4), on bacterial growth, fungal growth, and respiration rates (described below). For each salt the same molar concentrations were used (0, 0.06, 0.17, 0.52, 1.6, 4.7, 14.1, 42.3, 127, 380, 1,140, and 3,420 μmol salt per g soil). The resulting electrical conductivity in the soil-salt combinations was measured in a 1:5 soil-water suspension and covered a range of $EC_{1:5}$ values from 0.01 dS m^{-1} to 75 dS m^{-1}. Changes in soil pH following salt additions were small (from pH 6.4 to around 6.1 in the treatment receiving the highest-concentration salt addition). Measurements were repeated on fresh samples from the same soil to verify reproducibility. Bacterial growth measurements were repeated in three independent experiments, while fungal growth and respiration measurements were repeated in two independent experiments.

Bacterial and fungal growth. The bacterial growth rate was estimated by measuring the incorporation of ^3H-labeled leucine (Leu) into bacteria extracted from soil according to references 34 and 35. Two grams of soil was mixed with 20 ml of water, followed by a 10-min centrifugation step at $1,000 \times g$. From the resulting bacterial suspension a 1.5-ml subsample was used to measure bacterial growth. Two microliters of [^3H]Leu (37 MBq ml^{-1} and 5.74 TBq $mmol^{-1}$; PerkinElmer, United Kingdom) was added to the suspension together with nonlabeled Leu, resulting in a final concentration of 275 nM Leu. After 2 h of incubation at 22°C in the dark, bacterial growth was terminated by the addition of 100% trichloroacetic acid. After a series of washing steps (34), the amount of incorporated radioactive label was measured using liquid scintillation. To assess whether salt toxicity to bacterial growth rates could be underestimated by measuring bacterial growth in a 20-ml soil suspension, we varied the amount of water added to create the soil suspension (5, 10, 15, and 20 ml). When the salt concentration was considered on a per-gram-of-soil basis, the different dilutions associated with the homogenization/centrifugation step had no influence on the dose-response relationship (see Fig. S1 in the supplemental material). Since the volume of water added to the soil had no influence on the salt toxicity estimate (see Fig. S1), the estimated bacterial response was to the salt concentration in the soil, prior to water addition, rather than the concentration of salt in the bacterial suspension created.

Fungal growth was determined by measuring the incorporation of acetate (Ac) into ergosterol (32). One gram of soil was transferred into glass tubes to which a mixture of 20 μl [$1-^{14}C$]acetic acid (Na$^+$ salt; 2.04 GBq $mmol^{-1}$, 7.4 MBq ml^{-1}; PerkinElmer, United Kingdom) and unlabeled acetate was added together with 1.95 ml distilled water, resulting in a final acetate concentration of 220 μM. Samples then were incubated for 4 h at 22°C in the dark, after which growth was terminated with the addition of 1 ml 5% formalin. Ergosterol then was extracted, separated, and quantified using high-performance liquid chromatography and a UV detector (282 nm) and collected in a fraction collector (36). The radioactivity incorporated into the ergosterol fraction was measured using liquid scintillation.

Soil respiration. Basal soil respiration was measured by transferring 2 g of soil into a 20-ml vial and purging the headspace with pressurized air.

After purging, the vials were closed with crimp seals and incubated in the dark for approximately 16 h at 22°C. The CO_2 concentration in the headspace then was analyzed using a gas chromatograph (GC) equipped with a methanizer and a flame ionization detector, and background levels of CO_2 in pressurized air were subtracted.

Nitrogen transformation rates. Gross N mineralization and nitrification rates were determined using the ^{15}N pool dilution/enrichment technique [37]. Ten grams of soil was transferred to microcosms and mixed with 300 µl of NH_4Cl containing 0.24 g N liter^{-1} (16% ^{15}N). One set of soil samples was extracted with 50 ml 1 M KCl within a few minutes after the addition of the $^{15}NH_4^+$ label and a second set after an incubation period of 24 h at room temperature. The extract was filtered through a Whatman GF/F filter, and the concentrations of $^{14}NH_4^+$-N, $^{15}NH_4^+$-N, $^{14}NO_3^-$-N, and $^{15}NO_3^-$-N in the filtrate were determined according to standard procedures using acidified diffusion traps containing a filter disc [37]. The amounts of $^{15}N/^{14}N$ in the filter discs were measured at the stable isotope facility at the Department of Biology, Lund University, using a Flash 2000 elemental analyzer connected to a Delta V plus isotope-ratio mass spectrometer via the ConFlow IV interface (Thermo Scientific Inc., Bremen, Germany).

Duration of toxic effect. Since the different endpoints chosen typically are measured using different time frames, we also included assessments for the duration of toxic effect by the added salts. For bacterial growth and fungal growth, we covered a range of 2 h to 96 h after salt addition, and for the effects on mineralization (respiration), we covered a range of 6 h to 96 h.

Calculations and data analysis. In order to analyze the sensitivity of different microbial processes to salts, dose-response relationships were determined. To do this, values measured at different levels of salt addition first were normalized by dividing them by the average of the values measured in the samples that received no or low levels of salts and where no inhibition in process was observed. Normalized values then would fall in a range between 1 (no inhibition of process) and 0 (complete inhibition of process). Values obtained in repeated runs of the experiment were combined to generate a single curve. Log(IC_{50}) values (the logarithm of the salt concentration resulting in 50% inhibition of the process rate) were estimated using the logistic model $y = c/[1 + e^{b(x - a)}]$, where y is the relative normalized process rate, x is the logarithm of the salt concentration, a is the value of log(IC_{50}), b is a fitted parameter (slope) indicating the inhibition rate, and c is the process rate measured in the control without added salts [38]. Kaleidagraph 4.5.0 for Mac (Synergy Software, Reading, PA) was used to fit inhibition curves using this equation. To compare toxicity, 95% confidence intervals were estimated for the log(IC_{50}) values based on the logistic model. Our criterion for significant differences was nonoverlapping 95% confidence intervals. This is a conservative criterion, as nonoverlapping 85% confidence intervals correspond to an α of 0.05 [39] to determine statistical significance.

Gross N mineralization and nitrification rates were estimated by the ^{15}N pool dilution/enrichment technique [40–43] using the equations in Table S1 in the supplemental material.

RESULTS

Soil characteristics. The studied soil had a pH of 6.4 and an electrical conductivity of 0.09 dS m^{-1} (Table 1), classifying it as a nonsaline soil. The SOM content was 19 mg C g^{-1}, the NH_4^+ content was 3.26 µg N g^{-1}, and the NO_3^- content was 6.34 µg N g^{-1} (Table 1). The total amount of microbial biomass in the soil was 137 µg biomass C g^{-1}, of which 87 µg C g^{-1} was fungal and 50 µg C g^{-1} was bacterial biomass (Table 1).

Acute toxicity of NaCl to microbial processes. All processes investigated showed clear concentration-response relationships with salinity, with pronounced inhibition at high salt concentrations (Fig. 1 and 2). These concentration-response relationships could be described with a logistic model (R^2 values ranging from

TABLE 1 Overview of chemical and biological properties of the soil prior to salt exposure

Parameter	Mean (SE[a])
Water content (% dry wt)	19.6 (0.1)
Organic matter content (mg C g^{-1})	19.3 (0.1)
$EC_{1:5}$[b] (dS m^{-1})	0.09 (0.004)
pH[c]	6.4 (0.03)
N-NH_4^+ (µg g^{-1})	3.3 (0.20)
N-NO_3^- (µg g^{-1})	6.3 (0.18)
Total microbial biomass[d] (µg biomass-C g^{-1})	137 (8)
Fungal biomass[e] (µg biomass-C g^{-1})	87 (7)
Bacterial biomass[f] (µg biomass-C g^{-1})	50 (7)
Fungal growth (pmol Ac g^{-1} h^{-1})	14.6 (0.2)
Bacterial growth (pmol Leu g^{-1} h^{-1})	42 (2.6)
Respiration (µg CO_2-C g^{-1} day^{-1})	12.0 (1.1)
Gross N mineralization (µg N g^{-1} day^{-1})	1.4 (0.07)
Gross nitrification (µg N g^{-1} day^{-1})	2.0 (0.16)

[a] Standard errors of the means.
[b] Electrical conductivity in a 1:5 soil-water suspension.
[c] Measured in a 1:5 soil-water suspension.
[d] Based on substrate-induced respiration (SIR) measurement.
[e] Based on ergosterol concentration.
[f] Mass balance of total microbial biomass and fungal biomass.

0.78 to 0.95 with an average R^2 value of 0.88). The acute toxic effects for all of these processes remained unchanged in the interval 1 h to 48 h after salt addition (see Fig. S2 in the supplemental material). With a longer duration, bacterial growth started to recover between 48 and 96 h, while mineralization and fungal growth remained suppressed for the duration of this comparison. As such, the different standard time frames used for the different endpoints (2 h to 24 h) did not bias the outcome of the comparison. In addition, we also used soil samples sampled at different time points to investigate how robust our assessments were for generalization. A formal comparison of time points showed no differences between soil samples run at different time points. Log(IC_{50}) values estimated using the model ranged from 1.74 for fungal growth, corresponding to 54 µmol NaCl per g soil, to 2.94 for gross N mineralization, corresponding to 870 µmol NaCl per g (Table 2). If microbial responses were compared using the resulting electrical conductivity measured in the salt additions, log(IC_{50}) values ranged from 0.10 for gross nitrification, corresponding to 1.26 dS m^{-1}, to 0.96 for respiration, corresponding to 9.05 dS m^{-1} (Table 2).

Bacterial growth, fungal growth, and respiration were inhibited by NaCl exposure to a similar degree, with log(IC_{50}) values of 1.80, 1.74, and 1.90, corresponding to NaCl concentrations of 6.3, 5.5, and 7.9 mol liter^{-1} bacterial suspension or 63, 55, and 79 µmol NaCl per g soil, respectively. There is some indication that gross N mineralization (Fig. 2A), which had a log(IC_{50}) value of 2.94, was less sensitive to NaCl than nitrification [log(IC_{50}) = 1.96] (Fig. 2C). However, there were no significant differences between these processes in sensitivity to NaCl. Gross N mineralization was significantly less sensitive to NaCl than bacterial growth rates. Increasing salinity had no discernible effect on the ratio of C-to-N mineralization rates, which had an overall mass ratio of 9 ± 1.3 (means ± standard errors).

Comparative toxicities of salts. In the second part of the experiment, we compared the toxicities of different salts on bacterial and fungal growth as well as soil respiration (Table 2 and Fig. 3;
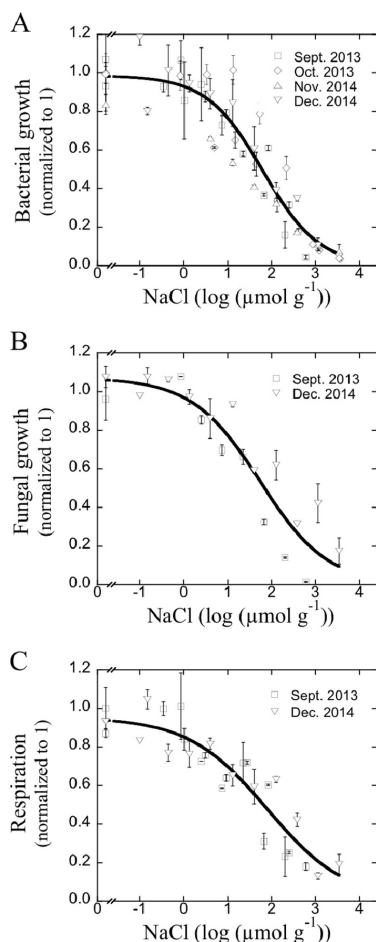
FIG 1 Concentration-response relationships between salt (NaCl) exposure and bacterial growth measured as leucine incorporation (A), fungal growth measured as acetate incorporation into ergosterol (B), and soil respiration (C). Soil samples were collected from the same site at different time points (represented by different symbols), and data from repeated runs of the experiment were combined into a single inhibition curve. Error bars indicate the standard errors ($n = 2$).
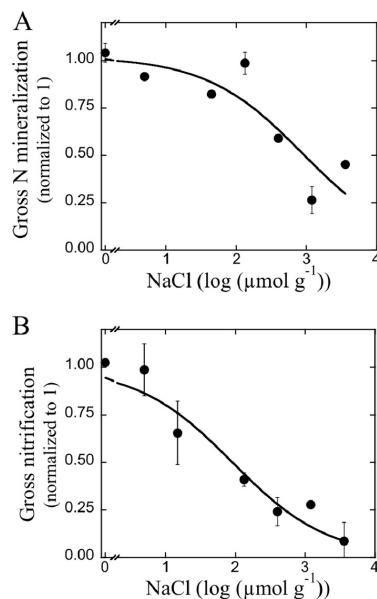

FIG 2 Concentration-response relationships between salt (NaCl) exposure and gross N mineralization (A) and gross nitrification (B). Error bars indicate standard errors ($n = 2$).

No significant differences between the susceptibility of bacterial growth to the different salts were found (Fig. 3A and Table 2). Fungal growth was significantly more inhibited by NaCl than any of the other studied salts (Fig. 3B and Table 3). KCl, Na_2SO_4, and K_2SO_4 did not differ in their effect on fungal growth rates. Of the salts included in the study, NaCl was the most inhibitory to respiration [$\log(IC_{50})$ of 1.90] (Fig. 3C and Table 2). There was no observable inhibitory effect of K_2SO_4 on respiration rates, even at concentrations that must have resulted in a saturation of the soil solution (Fig. 3C).

When salinities were expressed as electrical conductivities measured in 1:5 soil-water suspensions, results were, for the most part, similar to those using molar concentrations of salts (Table 3; also see Fig. S6 to S8 in the supplemental material). Of the few observed differences, we found that respiration was significantly less affected by NaCl exposure [$\log(IC_{50})$ of 0.96] than bacterial growth rates [$\log(IC_{50})$ of 0.26]. Respiration also was less affected by Na_2SO_4 [$\log(IC_{50})$ of 1.48] than both bacterial [$\log(IC_{50})$ of 0.05] and fungal [$\log(IC_{50})$ of 0.77] growth rates. In contrast to what was found using molar concentrations of salts, fungal growth rates were not more sensitive to NaCl than other salts (Table 3).

DISCUSSION

Microbial susceptibility to salts. Microbial growth responses, as well as respiration and N transformation rates, were clearly inhibited by salinity in our experiment. The toxic effects remained stable in the interval of 2 to 48 h after salt application, showing that the window of opportunity to compare toxic effects was rather wide, and the toxic effects were reproducible for repeated samplings of the same soil, between years and seasons, highlighting the

also see Fig. S3 to S5 in the supplemental material). While we could not observe any significant differences between bacterial growth, fungal growth, and respiration in response to NaCl exposure, we found that bacterial growth was more sensitive than fungal growth and respiration to KCl [$\log(IC_{50})$ of 1.43] and Na_2SO_4 [$\log(IC_{50})$ of 1.19] and K_2SO_4 [$\log(IC_{50})$ of 1.68] (Table 2). These $\log(IC_{50})$ values correspond to 2.7, 1.5, and 4.8 mol liter^{-1} bacterial suspension or 27, 15, and 48 mg g^{-1}, respectively. Fungal growth and soil respiration were affected by NaCl, KCl, and Na_2SO_4 to the same degree, while K_2SO_4 inhibited fungal growth [$\log(IC_{50})$ of 2.62, corresponding to 420 mg g^{-1}] but not respiration (Table 2).

TABLE 2 Sensitivity of microbial processes to exposure to NaCl, KCl, Na_2SO_4, and K_2SO_4

Microbial process	Sensitivity to[a]:							
	NaCl		KCl		Na_2SO_4		K_2SO_4	
	$Log(IC_{50})$	95% CI	$Log(IC_{50})$	95% CI	$Log(IC_{50})$	95% CI	$Log(IC_{50})$	95% CI
Growth[b]								
Bacterial	1.80 (0.10)	1.60–1.99	1.43 (0.21)	1.02–1.84	1.19 (0.17)	0.85–1.52	1.68 (0.29)	1.11–2.25
Fungal	1.74 (0.20)	1.34–2.13	2.53 (0.12)	2.30–2.77	2.56 (0.11)	2.34–2.77	2.62 (0.17)	2.29–2.96
Respiration	1.90 (0.18)	1.55–2.25	2.66 (0.16)	2.34–3.00	3.12 (0.19)	2.75–3.50	No inhibition	
Gross N mineralization	2.94 (0.36)	2.16–3.72						
Gross nitrification	1.96 (0.16)	1.61–2.31						

[a] IC_{50}s (measured as μmol salt g^{-1}) corresponding to the salt concentration leading to a 50% inhibition of process rate. Standard errors are given within parentheses. 95% CI is the 95% confidence interval of the IC_{50}.
[b] Bacterial growth was measured as [³H]leucine incorporation into biomass. Fungal growth was measured as [¹⁴C]acetate incorporation into ergosterol.

robustness of the assessments. The salinity responses of all microbial processes could be well described with a logistic dose-response relationship (Fig. 1 to 3). Our findings suggest that all of the above-described processes could be used as indicators for acute salinity effects on soil microorganisms and, as such, provide effective measures for comparative toxicity assessments.

In saline soil environments, changes in salinity of course would be more gradual than in our experiment. As a consequence, microbial communities have more time to respond to the changing conditions. Sensitive organisms would be outcompeted while tolerant organisms would thrive, resulting in a community change. These adaptations and community shifts could offset some of the inhibiting effect of salinity on the microbial community we document here. Nevertheless, microbial activity remains reduced in soils that experienced high salt concentrations for longer periods of time (12, 14, 20, 23), but it is often unclear to what degree this observed reduction represents a direct effect of salinity. Therefore, our results represent the potential susceptibility of a process to a direct salt exposure in the studied soil, while the degree to which microbial functions can recover from these perturbations remains to be investigated.

We found indications that fungi are more tolerant to acute salt exposure than bacteria for three out of four salts (Fig. 1 and Table 2), with the exception of NaCl (Table 2). It has been suggested that the chitinous cell walls of fungi offer better protection against water loss, which makes them more resistant to changes in moisture (6, 44). It would be reasonable that this also would offer protection against low water potential brought about by increased osmotic concentration. Consistent with this prediction, it has been shown that fungi are better able to cope with high osmotic pressure caused by high concentrations of organic substrates (8, 9). Moreover, the different localization of the proton gradient used for energy generation in prokaryotic and eukaryotic cells could make fungi more resistant to changes in the cation concentration outside the cell. In eukaryotic cells, postglycolytic reactions take place in the mitochondria, and the electrochemical potential used to drive ATP synthesis is established across the mitochondrial membrane, whereas in bacteria, the potential needs to be maintained across the cell membrane and therefore is more susceptible to environmental conditions outside the cell (45, 46).

The observation that fungal growth was less affected by acute salt exposure than bacteria suggests, if the finding can be extrapolated, that soil salinization favors fungi over bacteria, which

could result in a shift in community composition toward a more fungus-dominated community. In a recent study, an increase in the abundance of fungal biomarkers was observed in response to both increasing concentrations of salts and drying of the soil (47). However, important caveats for this extrapolation need to be carefully considered. The microcosm systems we used were experimentally dispersal limited, meaning that a number of halotolerant or halophilic microorganisms that would dominate the microbial community in naturally saline soils were not present in the nonsaline soil used in this study. While this would not affect the acute responses to salt, the recovery after salt exposure could have been greatly affected. It is possible that bacteria were particularly affected by this bias.

Previous literature reports on the relative dominance of fungi over bacteria in naturally saline soils could be used to evaluate how the acute toxicity responses of microorganisms can be translated to ecosystem effects. To date, these reports are scarce, however, and the few available studies do not unambiguously support the idea that saline soils become more fungus dominated. Saline soils generally have been found to contain low microbial biomass, often with a decreasing ratio of fungal to bacterial biomass (14–17). However, high salinity often coincides with high alkalinity, and it is possible that part of the observed negative dependence of fungi to increasing salinity is driven by the well-known effect of pH (32). Consistent with this, a recent study using a salinity gradient not confounded by soil pH differences observed higher fungal biomass and growth rates in highly saline soils than in nonsaline soils (13). While our knowledge on the effects of salinity is limited in soils, more systematic work is available for aquatic ecosystems. Although hypersaline aquatic environments are dominated by prokaryotes, halophilic aquatic fungi exist that also can grow under highly saline conditions (48). Additionally, eukaryotic decomposers may be underrepresented in most aquatic systems for reasons other than high salinity, e.g., the low availability of particulate organic substrate, which is more abundant in terrestrial environments.

Comparative toxicities of Cl^- and SO_4^{2-} salts. The inhibitory effect of high concentrations of salts on microbial processes is a combination of both the effects of highly negative osmotic potential and of specific ion toxicity. In the comparison of the toxicities of different salts, we have the opportunity to compare toxicity in terms of both total ionic strength (electric conductivity) and molar concentrations of added salts, thereby disentangling osmotic
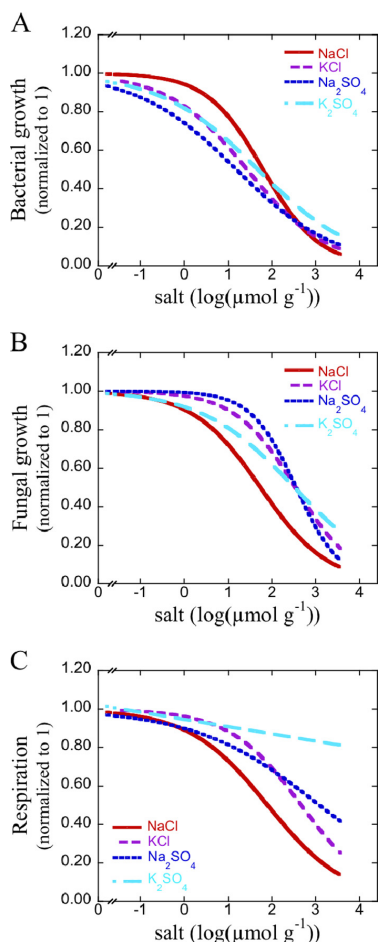
71

FIG 3 Dose-response relationships between bacterial growth (A), fungal growth (B), and soil respiration (C) and short-term exposure to different salts (NaCl, KCl, Na$_2$SO$_4$, and K$_2$SO$_4$). Data from repeated runs of the experiment were combined into a single inhibition curve. In the control treatments without added salt, the bacterial growth was 42 pmol Leu g^{-1} h^{-1}, the fungal growth rate was 15 pmol Ac g^{-1} h^{-1}, and the respiration rate was 1.8 μg CO$_2$ g^{-1} h^{-1}. Error bars indicate the standard errors ($n = 2$).

potential and specific ion toxicity. Our comparison suggests a lower toxicity of SO$_4^{2-}$ salts than of Cl$^-$ salts at a comparable ionic strength for respiration rates but not for microbial growth rates (Table 3).

High concentrations of salts in the cytoplasm of microbial cells can lead to enzyme inhibition due to salting out caused by high ionic strength. In addition, specific ion toxicities have been identified, e.g., some enzymes are particularly sensitive to Na$^+$ and Cl$^-$ due to interactions of the ions with inhibitory binding sites (49). A lower toxicity of SO$_4^{2-}$ ions than Cl$^-$ ions to soil microorganisms has been suggested previously (28), e.g., cultured rhizobial strains were found to be less affected by SO$_4^{2-}$ salts than the correspond-

ing Cl$^-$ salts (50). Chloride ions inside cells have been shown to inhibit protein synthesis by preventing the binding of ribosomes to mRNA (51, 52). SO$_4^{2-}$ ions, on the other hand, can be metabolized by many bacteria and fungi and have no ion-specific toxicity to cellular processes. We could not find clear differences between salts containing Na$^+$ and K$^+$ ions with regard to their toxicity to microbial processes, even though K$^+$ salts previously have been found to be less toxic than Na$^+$ salts (28).

Responses of the microbial growth efficiency to exposure to salts. Respiration was found to be less sensitive to exposure to SO$_4^{2-}$ salts than bacterial and fungal growth rates and was affected to a degree similar to that of fungal growth rates by Cl$^-$ salts. K$_2$SO$_4$ was not inhibitory to respiration rate and exerted only mild inhibitory effects on growth rates (Tables 2 and 3). This suggests that at high concentrations of SO$_4^{2-}$ salts, microorganisms still are actively respiring but no longer investing resources into biomass production, supporting the hypothesis that microorganisms allocate substrate away from biomass production toward maintenance functions in response to salt exposure, resulting in decreased MGE. At high concentrations of Cl$^-$ salts, in contrast, both growth and respiration were inhibited.

The methods we used to estimate microbial growth rates estimate protein production (bacterial growth) or lipid synthesis (fungal growth). As such, resources used for the synthesis of low-molecular-weight compounds for osmoregulation, such as organic solutes including betaine, ectoine, and various sugars and amino acids, would not be a form of growth captured by these methods. However, these physiological adjustments should affect estimates of MGE only to a minor degree (53). Lowered MGE of the microbial community in response to changes in environmental conditions is frequently interpreted as an indication of a stressed community (5, 54, 55). In our case, relating respiration to newly synthesized biomass would lead to the interpretation that exposure to SO$_4^{2-}$ salts is more stressful to the community than Cl$^-$ salt exposure. If these results of acute responses to salinity can be extrapolated to predict longer-term effects of salts in soil, they would suggest that the accumulation of SO$_4^{2-}$ salts in the soil can lead to a shift in C allocation from microbial anabolism to catabolism. However, this interpretation is problematically ambiguous. It is equally possible that Cl$^-$ salts actually exerted a stronger effect on microorganisms, leading to a higher rate of cell death than exposure to SO$_4^{2-}$ salts. The lower number of surviving cells could have resulted in a stronger inhibition of respiration together with growth, whereas during SO$_4^{2-}$ exposure more cells were still alive to respire. This would give the misleading impression of a more stressed community suggested by the reduced MGE, emphasizing that caution needs to be exercised in the interpretation and extrapolation of this endpoint.

Sensitivity of nitrification. Nitrification rates were not found to have a lower log(IC$_{50}$) value for salt exposure than the other studied microbial processes (Fig. 2C and Table 2). This contrasts with other studies where nitrification has been identified as a process that is especially sensitive to salinity (24, 56). An important difference between our study and many previous studies concerns the length of incubation after the addition of salts. We measured the acute toxicity of salts shortly after salt exposure, whereas other studies usually measure the response of nitrification and other processes after a longer incubation time. This renders our assessment more directly interpretable than previous assessments. In a longer-term assessment, the measured process is a product of two

TABLE 3 Sensitivity of microbial processes to the electrical conductivity in a 1:5 soil-water suspension following addition of NaCl, KCl, Na_2SO_4, and K_2SO_4

	Sensitivity to[a]:							
	NaCl		KCl		Na_2SO_4		K_2SO_4	
Microbial process	$Log(IC_{50})$	95% CI	$Log(IC_{50})$	95% CI	$Log(IC_{50})$	95% CI	$Log(IC_{50})$	95% CI
Growth[b]								
Bacterial	0.26 (0.10)	0.06 to 0.45	−0.09 (0.11)	−0.31 to 0.12	0.05 (0.10)	−0.14 to 0.25	0.51 (0.16)	0.20 to 0.83
Fungal	0.67 (0.15)	0.37 to 0.96	0.74 (0.17)	0.41 to 1.07	0.77 (0.11)	0.55 to 0.98	1.02 (0.24)	0.55 to 1.49
Respiration	0.96 (0.18)	0.60 to 1.31	0.68 (0.30)	0.06 to 1.30	1.48 (0.21)	1.06 to 1.89	No inhibition	
Gross N mineralization	0.94 (0.35)	0.18 to 1.70						
Gross nitrification	0.10 (0.15)	−0.23 to 0.43						

[a] IC_{50}s (measured as dS m^{-1}) corresponding to the electrical conductivity leading to a 50% inhibition of process rate. Standard errors are given within parentheses. 95% CI is the 95% confidence interval of the IC_{50}.
[b] Bacterial growth was measured as [^{3}H]leucine incorporation into biomass. Fungal growth was measured as [^{14}C]acetate incorporation into ergosterol.

components: first, the direct suppression or inhibition, and second, the recovery of the process due to sufficient time for a community shift with higher tolerance to salt to occur. Thus, the previous reports of higher sensitivity of nitrification than other processes could be a bias in the recovery of different processes rather than differences in initial tolerance. The steps involved in nitrification are carried out by only a small number of bacteria and archaea. After the addition of salts, microbes carrying out nitrification probably are affected to a degree similar to that of microbes carrying out other less specialized processes, reflected in a similar acute inhibition. However, a high functional redundancy allows alternative groups to quickly take over reduced general processes, such as respiration, thereby masking the inhibiting effect of salts. Conversely, nitrification remains impaired due to the small species pool of nitrifying organisms. Therefore, it appears that nitrification is a useful indicator of toxic effects of chemicals in recent history (57, 58), carrying a signal of inhibition for a long duration after exposure.

Conclusions. Our results show that salinity exerts a strong inhibitory effect on a range of microbial processes in soil, offering effective measures to assess comparative toxicity. Acute toxic effects of added salts occurred immediately (within 2 h) and lasted for at least 48 h before tolerance induced via community changes led to a recovery of process rates. Fungal growth was found to be less affected by salts than bacterial growth by three of the salts included in the study (KCl, Na_2SO_4, and K_2SO_4). This difference in tolerance could translate into ecological relevance by favoring fungi over bacteria at high salinities. Nitrification was not found to be more sensitive to exposure to salts than other processes, in contrast to previous findings, probably due to the longer experimental time frame in earlier assessments. Although salinity initially inhibits all microbial processes, the recovery of microbial processes with high functional redundancy, such as respiration, should be significantly faster than that of more specialized processes, such as nitrification, an imbalance that quickly would manifest as an apparent higher sensitivity of nitrification. All studied salts inhibited microbial growth rates to a similar degree, suggesting that the main factor affecting microbial growth rates is the total ionic strength of the soil solution. In contrast, respiration rates were affected less by salts containing SO_4^{2-} ions than Cl$^-$ salts, indicating specific respiration inhibition by Cl$^-$ ions. Respiration rates were inhibited less than microbial growth rates at the

same concentrations of SO_4^{2-} salts, which could lead to changes in MGE; however, alternative physiological interpretations stress that this index must be interpreted with caution.

REFERENCES

1. **Rengasamy P.** 2006. World salinization with emphasis on Australia. J Exp Bot **57:**1017–1023. http://dx.doi.org/10.1093/jxb/erj108.
2. **Martinez-Beltran J, Manzur CL.** 2005. Overview of salinity problems in the world and FAO strategies to address the problem, p 311–314. *In* Proceedings of the International Salinity Forum, Riverside, CA.
3. **Szabolcs I.** 1989. Salt-affected soils. CRC Press, Boca Raton, FL.
4. **Six J, Frey SD, Thiet RK, Batten KM.** 2006. Bacterial and fungal contributions to carbon sequestration in agroecosystems. Soil Sci Soc Am J **70:**555–569. http://dx.doi.org/10.2136/sssaj2004.0347.
5. **Manzoni S, Taylor P, Richter A, Porporato A, Ågren GI.** 2012. Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. New Phytol **196:**79–91. http://dx.doi.org/10.1111/j.1469-8137.2012.04225.x.
6. **Strickland MS, Rousk J.** 2010. Considering fungal:bacterial dominance in soils–methods, controls, and ecosystem implications. Soil Biol Biochem **42:**1385–1395. http://dx.doi.org/10.1016/j.soilbio.2010.05.007.
7. **Waring BG, Averill C, Hawkes CV.** 2013. Differences in fungal and bacterial physiology alter soil carbon and nitrogen cycling: insights from meta-analysis and theoretical models. Ecol Lett **16:**887–894. http://dx.doi.org/10.1111/ele.12125.
8. **Griffiths BS, Ritz K, Ebblewhite N, Dobson G.** 1999. Soil microbial community structure: effects of substrate loading rates. Soil Biol Biochem **31:**145–153.
9. **Reischke S, Rousk J, Bååth E.** 2014. The effects of glucose loading rates on bacterial and fungal growth in soil. Soil Biol Biochem **70:**88–95. http://dx.doi.org/10.1016/j.soilbio.2013.12.011.
10. **Manzoni S, Schimel JP, Porporato A.** 2012. Responses of soil microbial communities to water stress: results from a meta-analysis. Ecology **93:**930–938. http://dx.doi.org/10.1890/11-0026.1.
11. **Lennon JT, Aanerud ZT, Lehmkuhl BK, Schoolmaster DR.** 2012.

73

Mapping the niche space of soil microorganisms using taxonomy and traits. Ecology **93**:1867–1879. http://dx.doi.org/10.1890/11-1745.1.

12. **Wichern J, Wichern F, Joergensen RG.** 2006. Impact of salinity on soil microbial communities and the decomposition of maize in acidic soils. Geoderma **137**:100–108. http://dx.doi.org/10.1016/j.geoderma.2006.08.001.

13. **Kamble PN, Gaikwad VB, Kuchekar SR, Bååth E.** 2014. Microbial growth, biomass, community structure and nutrient limitation in high pH and salinity soils from Pravaranagar (India). Eur J Soil Biol **65**:87–95. http://dx.doi.org/10.1016/j.ejsobi.2014.10.005.

14. **Sardinha M, Müller T, Schmeisky H, Joergensen RG.** 2003. Microbial performance in soils along a salinity gradient under acidic conditions. Appl Soil Ecol **23**:237–244. http://dx.doi.org/10.1016/S0929-1393(03)00027-1.

15. **Pankhurst CE, Yu S, Hawke BG, Harch BD.** 2001. Capacity of fatty acid profiles and substrate utilization patterns to describe differences in soil microbial communities associated with increased salinity or alkalinity at three locations in South Australia. Biol Fertil Soils **33**:204–217. http://dx.doi.org/10.1007/s003740000309.

16. **Al-Busaidi KTS, Buerkert A, Joergensen RG.** 2014. Carbon and nitrogen mineralization at different salinity levels in Omani low organic matter soils. J Arid Environ **100**:106–110.

17. **Rasul G, Appuhn A, Müller T, Joergensen RG.** 2006. Salinity-induced changes in the microbial use of sugarcane filter cake added to soil. Appl Soil Ecol **31**:1–10. http://dx.doi.org/10.1016/j.apsoil.2005.04.007.

18. **Blagodatskaya E, Kuzyakov Y.** 2013. Active microorganisms in soil: critical review of estimation criteria and approaches. Soil Biol Biochem **67**:192–211. http://dx.doi.org/10.1016/j.soilbio.2013.08.024.

19. **Rousk J, Bååth E.** 2011. Growth of saprotrophic fungi and bacteria in soil. FEMS Microbiol Ecol **78**:17–30. http://dx.doi.org/10.1111/j.1574-6941.2011.01106.x.

20. **Setia R, Marschner P.** 2013. Impact of total water potential and varying contribution of matric and osmotic potential on carbon mineralization in saline soils. Eur J Soil Biol **56**:95–100. http://dx.doi.org/10.1016/j.ejsobi.2013.02.003.

21. **Chowdhury N, Nakatani AS, Setia R, Marschner P.** 2011. Microbial activity and community composition in saline and non-saline soils exposed to multiple drying and rewetting events. Plant Soil **348**:103–113. http://dx.doi.org/10.1007/s11104-011-0918-4.

22. **Laura RD.** 1974. Effects of neutral salts on carbon and nitrogen mineralization of organic matter in soil. Plant Soil **41**:113–127. http://dx.doi.org/10.1007/BF00017949.

23. **Rath KM, Rousk J.** 2015. Salt effects on the soil microbial decomposer community and their role in organic carbon cycling: a review. Soil Biol Biochem **81**:108–123. http://dx.doi.org/10.1016/j.soilbio.2014.11.001.

24. **Johnson D, Guenzi W.** 1963. Influence of salts on ammonium oxidation and carbon dioxide evolution from soil. Soil Sci Soc Am J **27**:663–666. http://dx.doi.org/10.2136/sssaj1963.03615995002700060028x.

25. **Darrah PR, Nye PH, White RE.** 1987. The effect of high solute concentrations on nitrification rates in soil. Plant Soil **97**:37–45. http://dx.doi.org/10.1007/BF02149821.

26. **McClung G, Frankenberger WT.** 1987. Nitrogen mineralization rates in saline vs salt-amended soils. Plant Soil **104**:13–21. http://dx.doi.org/10.1007/BF02370619.

27. **McClung G, Frankenberger WT.** 1985. Soil-nitrogen transformations as affected by salinity. Soil Sci **139**:405–411. http://dx.doi.org/10.1097/00010694-198505000-00005.

28. **Sindhu MA, Cornfield AH.** 1967. Comparative effects of varying levels of chlorides and sulphates of sodium, potassium, calcium and magnesium on ammonification and nitrification during incubation of soil. Plant Soil **27**:468–472. http://dx.doi.org/10.1007/BF01376343.

29. **Rasul G, Khan KS, Joergensen RG.** 2014. Microbial use of sugarcane filter cake in an artificial saline substrate varying in anion composition and inoculant at different temperatures. Arch Agron Soil Sci **60**:327–335. http://dx.doi.org/10.1080/03650340.2013.797571.

30. **Li X, Li F, Ma Q, Cui Z.** 2006. Interactions of NaCl and Na$_2$SO$_4$ on soil organic C mineralization after addition of maize straws. Soil Biol Biochem **38**:2328–2335. http://dx.doi.org/10.1016/j.soilbio.2006.02.015.

31. **Anderson JPE, Domsch KH.** 1978. Physiological method for quantitative measurement of microbial biomass in soils. Soil Biol Biochem **10**:215–221. http://dx.doi.org/10.1016/0038-0717(78)90099-8.

32. **Rousk J, Brookes PC, Bååth E.** 2009. Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineral-

ization. Appl Environ Microbiol **75**:1589–1596. http://dx.doi.org/10.1128/AEM.02775-08.

33. **Joergensen RG.** 2000. Ergosterol and microbial biomass in the rhizosphere of grassland soils. Soil Biol Biochem **32**:647–652. http://dx.doi.org/10.1016/S0038-0717(99)00191-1.

34. **Bååth E.** 1994. Thymidine and leucine incorporation in soil bacteria with different cell-size. Microb Ecol **27**:267–278.

35. **Bååth E, Pettersson M, Söderberg KH.** 2001. Adaptation of a rapid and economical microcentrifugation method to measure thymidine and leucine incorporation by soil bacteria. Soil Biol Biochem **33**:1571–1574. http://dx.doi.org/10.1016/S0038-0717(01)00073-6.

36. **Rousk J, Bååth E.** 2007. Fungal biomass production and turnover in soil estimated using the acetate-in-ergosterol technique. Soil Biol Biochem **39**:2173–2177. http://dx.doi.org/10.1016/j.soilbio.2007.03.023.

37. **IAEA.** 2001. Use of isotope and radiation methods in soil and water management and crop nutrition. IAEA, Vienna, Austria.

38. **Rousk J, Elyaagubi FK, Jones DL, Godbold DL.** 2011. Bacterial salt tolerance is unrelated to soil salinity across an arid agroecosystem salinity gradient. Soil Biol Biochem **43**:1881–1887. http://dx.doi.org/10.1016/j.soilbio.2011.05.007.

39. **Payton ME, Miller AE, Raun WR.** 2000. Testing statistical hypotheses using standard error bars and confidence intervals. Commun Soil Sci Plant Anal **31**:547–551. http://dx.doi.org/10.1080/00103620009370458.

40. **Blackburn TH.** 1979. Method for measuring rates of NH$_4^+$ turnover in anoxic marine-sediments, using a ^{15}N-NH$_4^+$ dilution technique. Appl Environ Microbiol **37**:760–765.

41. **Bengtson P, Falkengren-Grerup U, Bengtsson G.** 2006. Spatial distributions of plants and gross N transformation rates in a forest soil. J Ecol **94**:754–764. http://dx.doi.org/10.1111/j.1365-2745.2006.01143.x.

42. **Nishio T, Kanamori T, Fujimoto T.** 1985. Nitrogen transformations in an aerobic soil as determined by a ^{15}NH$_4^+$ dilution technique. Soil Biol Biochem **17**:149–154. http://dx.doi.org/10.1016/0038-0717(85)90106-3.

43. **Wessel WW, Tietema A.** 1992. Calculating gross N transformation rates of ^{15}N pool dilution experiments with acid forest litter–analytical and numerical approaches. Soil Biol Biochem **24**:931–942. http://dx.doi.org/10.1016/0038-0717(92)90020-X.

44. **Holland EA, Coleman DC.** 1987. Litter placement effects on microbial and organic matter dynamics in an agroecosystem. Ecology **68**:425–433. http://dx.doi.org/10.2307/1939274.

45. **Russell JB, Sharp WM, Baldwin RL.** 1979. Effect of pH on maximum bacterial growth rate and its possible role as a determinant of bacterial competition in the rumen. J Anim Sci **48**:251–255.

46. **Garland PB.** 1977. Energy transduction in microbial systems. Symp Soc Gen Microbiol **27**:1–21.

47. **Kakumanu ML, Williams MA.** 2014. Osmolyte dynamics and microbial communities vary in response to osmotic more than matric water deficit gradients in two soils. Soil Biol Biochem **79**:14–24. http://dx.doi.org/10.1016/j.soilbio.2014.08.015.

48. **Gostinčar C, Lenassi M, Gunde-Cimerman N, Plemenitaš A.** 2011. Fungal adaptation to extremely high salt concentrations. Adv Appl Microbiol **77**:71–96. http://dx.doi.org/10.1016/B978-0-12-387044-5.00003-0.

49. **Serrano R.** 1996. Salt tolerance in plants and microorganisms: toxicity targets and defense responses. Int Rev Cytol **165**:1–52. http://dx.doi.org/10.1016/S0074-7696(08)62219-6.

50. **Elsheikh EAE, Wood M.** 1989. Response of chickpea and soybean rhizobia to salt–osmotic and specific ion effects of salts. Soil Biol Biochem **21**:889–895. http://dx.doi.org/10.1016/0038-0717(89)90077-1.

51. **Choquet CG, Kamekura M, Kushner DJ.** 1989. *In vitro* protein-synthesis by the moderate halophile *Vibrio costicola*–site of action of Cl$^-$ ions. J Bacteriol **171**:880–886.

52. **Weber LA, Hickey ED, Maroney PA, Baglioni C.** 1977. Inhibition of protein-synthesis by Cl$^-$. J Biol Chem **252**:4007–4010.

53. **Neidhardt FC.** 1999. Bacterial growth: constant obsession with dN/dt. J Bacteriol **181**:7405–7408.

54. **Anderson TH, Domsch KH.** 1993. The metabolic quotient for CO$_2$ (qCO_2) as a specific activity parameter to assess the effects of environmental conditions, such as pH, on the microbial biomass of forest soils. Soil Biol Biochem **25**:393–395. http://dx.doi.org/10.1016/0038-0717(93)90140-7.

55. **Wardle DA, Ghani A.** 1995. A critique of the microbial metabolic

quotient (qCO$_2$) as a bioindicator of disturbance and ecosystem development. Soil Biol Biochem **27:**1601–1610. http://dx.doi.org/10.1016/0038-0717(95)00093-T.

56. **Greaves JE.** 1922. Influence of salts on bacterial activities of soil. Bot Gaz **73:**161–180. http://dx.doi.org/10.1086/332973.

57. **Pell M, Stenberg B, Torstensson L.** 1998. Potential denitrification and nitrification tests for evaluation of pesticide effects in soil. Ambio **27:** 24–28.

58. **Hansson GB, Klemedtsson L, Stenstrom J, Torstensson L.** 1991. Testing the influence of chemicals on soil autotrophic ammonium oxidation. Environ Toxicol Water Qual **6:**351–360. http://dx.doi.org/10.1002/tox.2530060307.

Inducing community tolerance to salt in soil microbial communities in a microcosm experiment

Rath Kristin M[1,2], Maheshwari Arpita[1] and Rousk Johannes[1]

[1] Section of Microbial Ecology, Department of Biology, Lund University, Lund, Sweden

[2] Centre for Environmental and Climate Research (CEC), Lund University, Lund, Sweden

Abstract

Soil salinity is an environmental problem affecting an increasing land area globally. High salt concentrations reduce microbial growth and respiration and microorganisms need to adapt to changing salinity to counteract its negative effect. We studied the ability of microbial communities to adapt to increased salt concentrations in soil by adjusting a non-saline soil to four different salinities through addition of NaCl (0, 2, 7 and 22 mg NaCl g^{-1}) and monitored the development of community salt tolerance over time. We also connected this information to changes in the community composition. In addition we assessed the change of respiration and bacterial and fungal growth over time as communities adapted to salinity. Community salt tolerance was induced quickly within one week and higher salt additions resulted in higher tolerance to salt and the induced tolerance mapped well onto bacterial community changes. Respiration decreased with increasing salinity. Bacterial and fungal growth were not clearly related to salinity, instead their response to salinity seemed to be modified by interactions between the two groups. Fungal growth was highest when bacterial growth was suppressed by salinity in the highest salt treatment, and lowest in the treatment with the highest bacterial growth rate. The fact that fungi grew better in the highest salt treatment (22 mg NaCl g^{-1}) than bacteria corroborates a higher fungal tolerance of salt. In conclusion, community salt tolerance can be induced quickly in soil microbial communities, even if they had not been previously exposed to high salt concentrations.

1. Introduction

Large areas of land are globally affected by high salt concentrations in soil (Rengasamy, 2006). Through land use change and mismanagement of land used for irrigation agriculture the area of salt-affected soils is increasing as evaporating

groundwater or water used for irrigation leaves behind dissolved salts, which leads to accumulation of salts in the topsoil (Jobbagy and Jackson, 2004; FAO, 2011). Soil microorganisms are negatively affected by high salt concentrations, which is reflected in e.g. decreased respiration and growth after salt exposure (Setia et al., 2011; Rath and Rousk, 2015; Rath et al., 2016). However, microorganisms can counteract some of the negative effects of salinity through physiological adaptations. These adaptions include the synthesis of osmolytes (Kakumanu and Williams, 2014) and changes in the composition of cell membranes (Turk et al., 2007; Zhang and Rock, 2008). Together with shifts in the community composition towards a community composed of more salt-tolerant species these physiological adaptations manifest as an increased community salt tolerance in response to salinity. As the community tolerance increases, microbial process rates that were inhibited in response to acute salt exposure could recover. An increased community salt tolerance with higher soil salinity has been found along natural gradients of salinity (Kamble et al., 2014, Paper IV).

In saline soils, microbial communities are often subject to fluctuations in salt concentrations, with salt concentrations increasing as soils dry out (Zhang et al., 1999; Tripathi et al., 2006). As salt concentrations rise, microbial communities would have to change their tolerance to salt quickly to adapt to the changing conditions, either through physiological changes or by replacement of species. It is unknown at which time-scales microbial communities can adjust to salinity and increase their resistance to salt to a level that matches the new salinity.

Fungi and bacteria are reported to be differently affected by salt exposure (Kamble et al., 2014; Rath et al., 2016). Generally, fungi appear to be more resistant to short-term exposure to salinity (Rath et al., 2016). However, it is unclear, whether an increased resistance to short-term exposure would indeed translate into a shift towards a more fungal-dominated system, as both increasing fungal (Wichern et al., 2006; Kamble et al., 2014) and increasing bacterial dominance (Pankhurst et al., 2001; Sardinha et al., 2003) in response to high soil salinity have been reported. While bacteria and fungi fulfill similar roles as decomposers of organic matter they differ in the range of substrates they can decompose (de Boer et al., 2005). Fungal and bacterial biomass also differ in their chemical composition (Six et al., 2006) and nutrient content (Mouginot et al., 2014). Thus shifts in the relative contribution of fungi and bacteria in response to salinity could have implications for C and nutrient dynamics in soil (Strickland and Rousk, 2010; Schmidt et al., 2011).

In this study we adjusted a non-saline soil to four different salinities in a microcosm setup. The aim was (a) to assess the time scale at which community salt tolerance increases in response to salt exposure, (b) to link increasing community salt tolerance to bacterial community structure, (c) to monitor respiration and bacterial and fungal growth as microbial communities adapted to salinity and (d) study whether bacteria or fungi were favored by increasing salinity

in the system. We hypothesized that community salt tolerance would be able to adjust to salinity and that final community salt tolerance would be reached at a later time point in more saline treatments. We also predicted that the community salt tolerance would increase driven by shifts in the community. Our prediction was that respiration and growth would be initially low in the saline treatments, but able to recover due to induced community tolerance driven by a structural change in the community. However, we expected respiration and growth to remain lower overall in high salinity treatments compared to low salinity treatments. We also hypothesized that bacteria would perform worse at higher salinities than fungi, and therefore bacterial growth would decrease more strongly with increasing salinity than fungal growth.

2. Material and methods

2.1. Soil

Soil was collected in May 2015 from a grassland site in Vomb, Southern Sweden (55° 40' 27" N, 13° 32' 45" E). Multiple samples were collected from a depth of 0-20 cm and combined into a composition soil sample. Roots were removed and the soil samples was sieved (<2.8 mm). The water content of the soil (gravimetric, 24 h at 105 °C) was ca. $28 \pm 0.4\%$ dry weight (dw) (mean $\pm$ 1SE of three replicates), a water holding capacity (WHC) of 65 ± 2 % dw and an organic matter content (loss on ignition, 600°C for 12 h) of 19.6 ± 0.6 % dw. In a 1:5 soil:water mixture the pH was 6.1 ± 0.02 and the electrical conductivity (EC) was measured as 0.09 ± 0.005 dS m^{-1}.

2.2. Experimental setup

Microcosms were setup with 250 g of soil in 1 l plastic containers with an airtight lid and adjusted to four different levels of salinity by adding different amounts of NaCl (0, 2, 7 and 22 mg NaCl g^{-1} soil) together with 100 µl of water per g soil. These addition resulted in electrical conductivities ranging from 0.1 dS m^{-1} to 7 dS m^{-1} (Table 1). Duplicate microcosms were prepared for each treatment. Microcosms were supplied with 15 mg g^{-1} soil 1:1 ground alfalfa:wheat straw mixture. Over the course of the experiment (40 days) microcosms were incubated at 18°C in the dark. The water content in the microcosms was monitored and maintained at a constant level, and microcosms were regularly aerated. On days 1, 2, 3, 5, 7, 10, 15, 20, 26 and 40 after salt addition, respiration, bacterial growth and fungal growth were measured in subsamples from each microcosm. In addition, community tolerance was estimated (see section 2.6.). Soil samples for the determination of bacterial community structure were taken at the end of the experiment on day 40.

Table 1:

Salinity of the four different salt addition treatments

Treatment	NaCl addition (mg g^{-1})	EC[1] (dS m^{-1})
I	0	0.1
II	2	1.1
III	7	2.8
IV	22	6.8

[1] Electrical conductivity in a 1:5 soil:water mixture

2.3. Bacterial growth

Bacterial growth was measured as the incorporation of ^{3}H-labelled leucine into bacterial biomass in bacteria extracted from soil according to Bååth (1994) and Bååth et al. (2001). Briefly, soil suspension was created by mixing 1 g of soil with 20 ml of water followed by a centrifugation at 1000 g. From the suspension 1.35 ml of suspension was taken. ^{3}H-labelled leucine (2µl; 37 MBq ml−1 and 5.74 TBq mmol−1; Perkin Elmer, UK) was added to the suspension together with non-labeled leucine, resulting in a final concentration of 275 nM Leu. After 1 h incubation at 18°C in the dark, growth was terminated by the addition of 100% tri-chloroacetic acid. After a series of washing steps, the amount of incorporated label was measured using liquid scintillation.

2.4. Fungal growth

Fungal growth was estimated using the incorporation of ^{14}C-labelled acetate into ergosterol according to Bååth (2001). Briefly, 1g of soil was mixed with 2 ml of water to create a soil slurry. To the soil slurry, 20 µl of 1-([^{14}C] acetic acid (sodium salt, 37 MBq ml^{-1}, 2.10 GBq mmol^{-1}, Perkin Elmer) were added combined with 30 µl 16 mM non-labelled sodium acetate, resulting in a final concentration of ca. 220 µM of sodium acetate. Samples were incubated at 18°C in the dark for 4 h, after which growth was terminated using 1 ml of 5% formalin. Ergosterol was then extracted from the samples and separated using high-performance liquid chromatography and a UV detector, and collected in a fraction collector. The radioactivity in the sample was measured using liquid scintillation.

2.5. Respiration

Portions of soil (1g) were weighed into 20 ml glass vials, purged with pressurized air, sealed and incubated for ca. 16 h at 18°C in the dark. Afterwards the CO_2 concentration in the headspace was analyzed using gas chromatograph (GC), equipped with a methanizer and a flame ionization detector, and background levels of CO_2 in pressurized air were subtracted.

2.6. Salt tolerance estimations

Community salt tolerance was estimated by measuring the inhibition of bacterial growth at different salinities in soil suspension. Soil suspensions were created as described in section 2.3. From each sample, subsamples of 1.35 ml suspension were taken and mixed with 0.15 ml of different NaCl solutions with concentrations of 0, 0.008, 0.02, 0.07, 0.22, 0.67, 2.0 and 6.0 mol/l of NaCl. Afterwards bacterial growth was measured as described in section 2.3.

2.7. Soil bacterial community composition

The composition of the bacterial community was determined through sequencing of the bacterial 16S rRNA gene. DNA was extracted from portions of 250 mg of homogenized ground soil using the MoBio PowerSoil DNA Isolation Kit (Carlsbad, CA, USA) according to the manufacturer's recommendations. Extracted DNA was amplified using the 16S rRNA gene primer pair 515-F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806-R (5′-GGACTACHVGGGTWTCTAAT-3′), which included Illumina adapters and unique barcode sequences for each sample. PCR was performed with GoTaq® Hot Start PCR Master Mix (Promega, Madison, WI USA) in a 25 μL reaction. Thermal cycling consisted of an initial denaturation step at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C (45s), annealing at 50°C (30 s), extension at 70°C (90 s), and a final extension at 72°C for 10 min. The amplified DNA was sequenced using a Illumina MiSeq platform (Illumina, San Diego, CA USA).

Sequences were processed using the UPARSE pipeline (Edgar, 2013) as described in Ramirez et al. (2014). Sequences were quality filtered and clustered de novo into operational taxonomic units (OTUs) at a 97% similarity level. Taxonomic information was assigned to OTUs using the 16S rRNA Greengenes database (McDonald et al., 2012). To correct for differences in sequencing depth samples were rarefied to 10 000 reads. OTUs that were observed fewer than 10 times across all samples were excluded from downstream analyses.

2.8. Data analysis

Bacterial growth rates at the eight different concentrations of NaCl used to estimate salt tolerance were normalized to the highest rates measured in each sample. Dose-response relationships were established between the logarithm of NaCl concentration in the suspensions and normalized bacterial growth using a logistic model (Rousk et al., 2011), $Y = c/[1 + e^{b(x-a)}]$, where Y is the leucine incorporation rate, x is the logarithm of the salt concentration in the suspension, a is the IC_{50}, c is the bacterial growth rate in the control without added salt, and b is a slope parameter indicating the rate of inhibition. The IC_{50} denotes the salt

concentration at which bacterial growth is inhibited by 50% compared to the growth measured without adding further NaCl in the suspension.

Shifts in the bacterial community composition were visualized using principal coordinate analysis (PCoA) based on Bray-Curtis dissimilarities after Hellinger transformation of data. The first axis of the PCoA was then regressed against community salt tolerance. A Mantel test with 1000 permutations was performed using Spearman's correlation coefficient to test for a significant correlation between the community composition and community salt tolerance. OTUs with an abundance of >0.5% of reads in at least one sample were correlated with community salt tolerance using Spearman's correlation to identify abundant OTUs positively correlated ($\rho > 0.5$) with community salt tolerance. Statistical analyses of the bacterial community composition were carried out in the R environment using the *vegan* package (Oksanen et al., 2016).

3. Results

3.1. Bacterial salt tolerance over time

The IC_{50} of different treatments started with similar values corresponding to 100 to 200 mM NaCl in suspension in all treatments on day 0 (Fig. 1B). However, there was high variability in the measurements of bacterial salt tolerance during the first 3 days of the experiment. From ca. day 10 onwards IC_{50} values were clearly lower in the 0 and 2 mg NaCl g^{-1} treatment than in the 7 and 22 mg NaCl g^{-1}. The IC_{50} in the 0 mg NaCl g^{-1} treatment fluctuated around a level corresponding to 100 mM NaCl and in the 2 mg NaCl g^{-1} treatment around a level of 200 mM NaCl. The IC_{50} in the 7 mg NaCl g^{-1} treatment reached a level corresponding to ca. 300 mM NaCl on day 10 and remained at that level after that. In the 22 mg NaCl g^{-1} treatment the IC_{50} increased until a level of ca. 650 mM NaCl on day 20. Overall, bacterial salt tolerance increased with increasing salinity of the treatment (Fig. 1C).
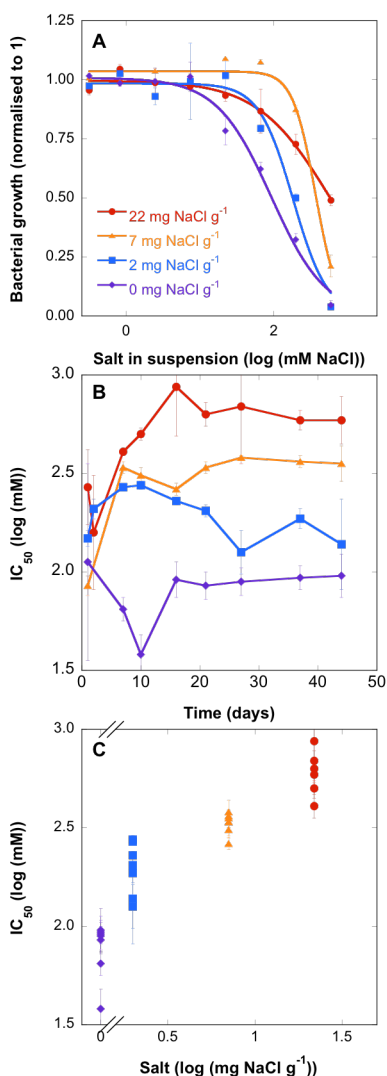
Fig. 1
Dose-response curves between salt concentration and bacterial growth at day 40 after salt addition. Bacterial growth was normalized to the maximum rate for each treatment. (B) IC_{50} for bacterial growth in 4 different salt treatments (0, 2, 7 and 22 mg NaCl g^{-1}) over time. (C) IC_{50} for bacterial growth from day 10 against salinity in different treatments.

3.2. Bacterial community responses to salt exposure

At the end of the experiment, salt exposure had resulted in pronounced differences in community composition between treatments (Fig. 2). In a principal coordinate analysis, the first ordination axis accounted for 40% of the variation of the dataset and the second ordination axis accounted for 35% of the variation (Fig.2). The four different salt treatments resulted in distinct communities, with similar community composition in duplicates of the same treatment. An arch effect was visible in the ordination. The first PC could be related to community salt tolerance (Fig. 2). In a Mantel test, bacterial community composition and community salt tolerance were significantly correlated with each other ($\rho = 0.61$, $p < 0.01$). Among the taxa that were correlated with increasing community salt tolerance (Spearman's $\rho > 0.5$) were primarily Firmicutes (Table S1). Out of the 17 OTUs correlated with community salt tolerance that had a maximum abundance of >0.5%, 9 belonged to the phylum Firmicutes, while the rest included members of the Gammaproteobacteria, Actinobacteria, Bacteroidetes and Verrucomicrobia.
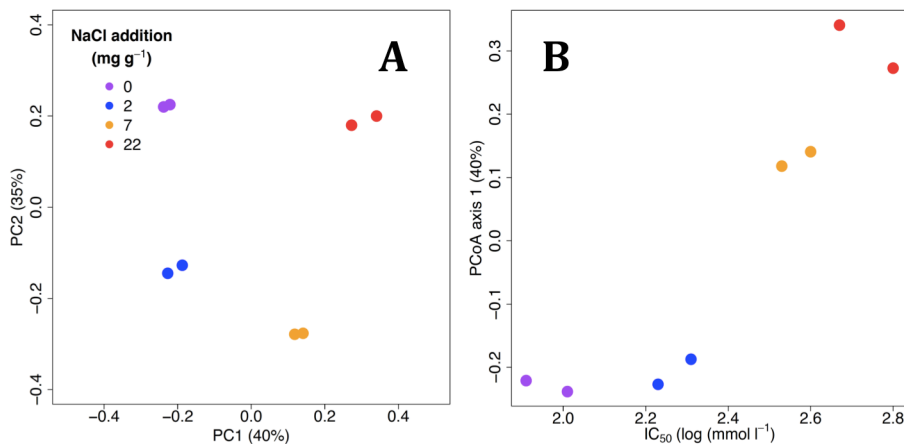
Fig. 2
(A) Principal coordinate analysis (PCoA) of bacterial community composition based on Bray-Curtis dissimilarities of 16S rRNA amplicon sequencing data in four different salt treatments (0, 2, 7 and 22 mg NaCl g^{-1}). (B) The first ordination axis of the PCoA against community salt tolerance indicated by the IC_{50}.

3.3. Respiration and microbial growth

In the 0 and 2 mg NaCl g^{-1} treatment respiration was highest on the first day of the experiment and then decreased exponentially over the course of the next 40 days (Fig. 3A). On day 1 respiration was higher in the 0 mg NaCl g^{-1} than in the 2 mg NaCl g^{-1} treatment, but later on respiration rates were similar in the two treatments. In the 7 mg NaCl g^{-1} and the 22 mg NaCl g^{-1} treatments respiration was low initially and reached its highest rate on days 2 and 3, respectively. Afterwards the respiration rate declined exponentially. At the end of the experiment on day 40 respiration was lower in the 22 mg NaCl g^{-1} treatment than in the other treatments. Cumulative respiration decreased with increasing salinity from ca. 250 µg CO_2 g^{-1} soil in the 0 mg NaCl g^{-1} treatment to ca. 100 µg CO_2 g^{-1} soil in the 22 mg NaCl g^{-1} treatment (Fig. 4A).
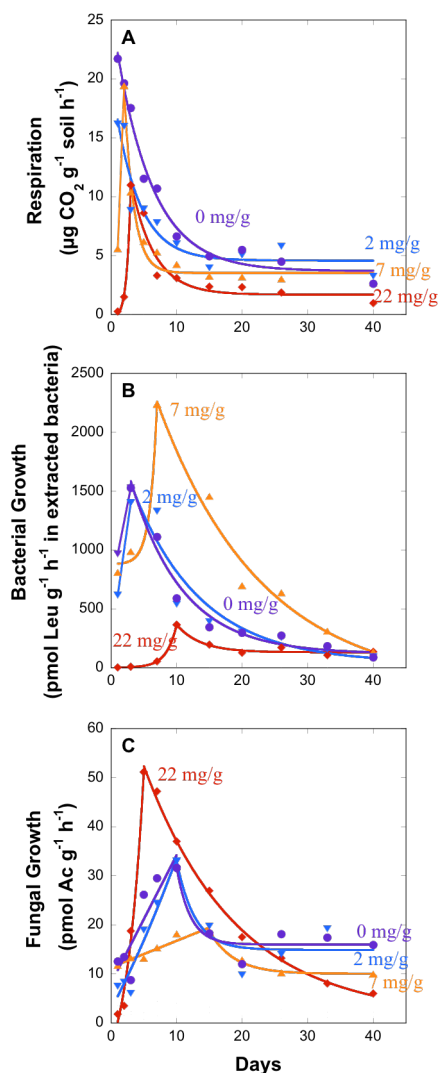
Fig. 3
Respiration (A), bacterial growth (B) and fungal growth rates (C) in microcosms of four different salt treatments (0, 2, 7 and 22 mg NaCl g^{-1}) with time after salt addition.

Bacterial growth rate in the 0 and 2 mg NaCl g^{-1} treatments peaked at day 2 and decreased exponentially afterwards (Fig. 3B). In the 7 mg NaCl g^{-1} treatment bacterial growth rates were similar to those in the 0 and 2 mg NaCl g^{-1} treatments on day 1, but then increased sharply until day 10. This was followed by an exponential decrease, during which bacterial growth rates in the 7 mg NaCl g^{-1} treatment remained higher than in any other treatment throughout most of the experiment. In the 22 mg NaCl g^{-1} treatment bacterial growth rates were close to 0 in the beginning of the experiment (Fig. 3B). Bacterial growth in the 22 mg NaCl g^{-1} treatment then recovered slightly until day 10, but never reached rates as high as the peak growth rates in the other treatments. On day 40, bacterial growth rates were similar in all treatments. Cumulative bacterial growth in the 7 mg NaCl g^{-1} treatment was much higher than in any other treatments, with a 300% increase over the cumulative growth in the 0 and 2 mg NaCl g^{-1} treatments (Fig. 4B). Cumulative bacterial growth was lowest in the 22 mg NaCl g^{-1} treatment.

Fungal growth rates took longer to increase to their maximum rates than both respiration and bacterial growth (Fig. 3C). Fungal growth rates in the 0 and 2 mg NaCl g^{-1} treatment were similar for much of the experiment. A linear increase was seen until day 10, followed by a decrease. On day 0 fungal growth in the 7 mg NaCl g^{-1} treatment was similar to the 0 mg NaCl g^{-1} treatment, but afterwards the increase of fungal growth in the 7 mg
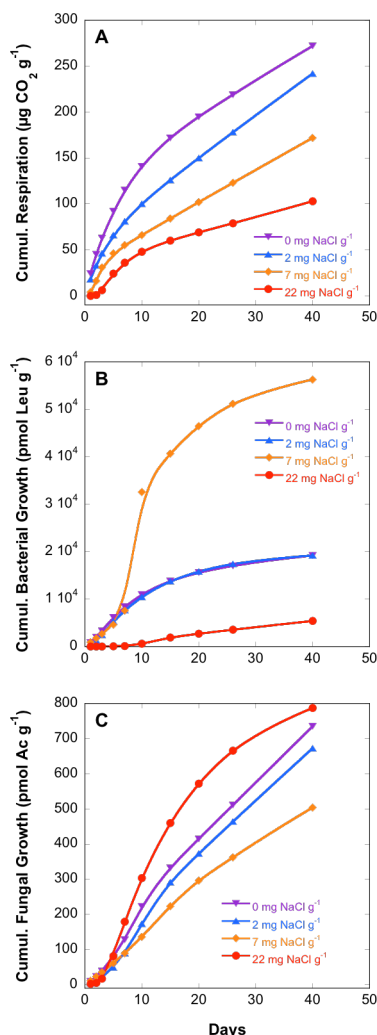
Fig.4
Cumulative respiration (A), bacterial growth (B) and fungal growth rates (C) in microcosms of 4 different salt treatments (0, 2, 7 and 22 mg NaCl g^{-1}) with time after salt addition.

NaCl g^{-1} treatment was low. The maximum rate in the 7 mg NaCl g^{-1} treatment was measured on day 15, and was lower than the maximum fungal growth in any other treatment. In the 22 mg NaCl g^{-1} treatment fungal growth was initially close to 0, but then increased rapidly until day 10, followed by an exponential decrease. On day 40, fungal growth was highest in the 0 and 2 mg NaCl g^{-1} treatments, and lowest in the 22 mg NaCl g^{-1} treatment. Cumulative fungal growth was highest in the 22 mg NaCl g^{-1} treatment and lowest in the 7 mg NaCl g^{-1} treatment (Fig. 4C).

4. Discussion

4.1. Induced bacterial community tolerance to salt and bacterial community composition

As expected, salt exposure induced increased community salt tolerance in all treatments with added salt (Fig. 1). The level of salt tolerance increased with the amount of salt that communities were exposed to (Fig. 1). Adaptation was rapid and occurred within only a few days after salt exposure, which suggests that microbial communities can quickly respond to changing environmental conditions. After a few days, salt tolerance did not increase further, as the level of salt tolerance that was reached was appropriate for the salinity in the treatment (Fig. 1B). It took about 10 days longer in the 22 mg NaCl g^{-1} treatment to reach a constant level of community salt tolerance than in the less saline treatments. It has to be kept in mind that in this study communities were supplied with ample resources through the addition of plant material. Since adaptation mechanisms require both energy and C-resources for the synthesis of e.g. compatible osmolytes (Oren, 1999; Kakumanu and Williams, 2014), it is possible that under resource limitation induced community tolerance to salt would

develop more slowly. Despite a relatively minor impact of salinity on respiration and growth in the 2 mg NaCl g^{-1} treatment (Fig. 3,4), increased community salt tolerance was induced also in this treatment. Together with increased community tolerance, bacterial community composition was changed as well (Fig. 2). Environmental factors can act upon microbial communities and change the composition of the community, without manifesting in altered process rates, as microbial communities have a high degree of functional redundancy (Berga et al., 2017).

As the soil used in this experiment had no prior history of salinization, it is likely that the species pool contained few or no salt-tolerant species compared to a community that had been exposed to high salinity previously (Vass and Langenheder, 2017). This starting pool of species could have limited the degree of salt tolerance achievable in the community. In addition, in natural systems dispersal could bring in more salt-adapted species from surrounding localities, promoting the establishment of a more salt-tolerant local community (Shade et al., 2012). In the 2 and 7 mg NaCl g^{-1} treatment the limitations of the species pool and the lack of dispersal were not limiting to the development of community salt tolerance, since communities could adapt to the even higher salinity in the 22 mg NaCl g^{-1} (Fig. 1). However, it is possible that at similar salinities to those reached in the 22 mg NaCl g^{-1} treatment, higher community salt tolerance could have been achieved with a different species pool (Paper IV). Along natural salinity gradients, salt community tolerance in soils of similar salinities as indicated by the IC$_{50}$ was found to be about a factor of 1.5 to 2.5 higher, ranging between ca. 1000 mM to 1500 mM NaCl in suspension (Paper IV), compared to the IC$_{50}$ of 650 mM in the 22 mg NaCl g^{-1} microcosms.

Clear differences in bacterial community composition between salt treatments had developed by the end of the experiment, suggesting that much of the changes in salt tolerance of the bacterial community where connected to shifts in community composition, with more salt-tolerant species replacing less salt-tolerant ones. The arch effect visible in the ordination is a common phenomenon in ordination methods when species gradually replace each other along environmental gradients (James and Mcculloch, 1990). Many of the bacterial OTUs positively linked to increased community salt tolerance belonged to the phylum Firmicutes. The Firmicutes include many species that form endospores and are thus able to survive in extreme environments, among those many species known to be salt-tolerant (Horikoshi et al., 2011). The ability to form spores would have given Firmicutes an advantage to survive the acute effects of salt exposure and grow more abundant after other bacteria had died off.

4.2. Respiration response to salt exposure

Cumulative respiration decreased with increasing salinity as expected (Fig. 4A). Decreasing respiration rates are a common functional response in salt-affected soils (Chowdhury et al., 2011; Hasbullah and Marschner, 2015; Rath and Rousk, 2015). In the less saline treatments, plant substrate fuelled high respiration rates on the first day after addition, whereas in the treatments receiving 7 and 22 mg NaCl g^{-1} respiration rates were lower on the first day and reached their maximum rates in the following days (Fig. 3A). The time before respiration reached its peak rate increased with increasing salinity (Fig. 3A). In the 22 mg NaCl g^{-1} treatment respiration was initially close to 0, indicating that mineralization of the added plant material was almost completely inhibited in the beginning. As salt was added to the saline treatments a part of the microbial community would have become inactive or died, and replaced by better adapted species. A smaller surviving microbial biomass in the saline soil with increasing salinity of the treatment then manifested itself in reduced mineralization of plant material.

Respiration increased more rapidly after addition of substrate than bacterial and fungal growth (Fig. 3), which means that added substrate was initially used for respiration rather than the build-up of new biomass. The discrepancy between the onset of respiration and the onset of growth was especially apparent in the treatments receiving ≥ 7 mg NaCl g^{-1} (Fig. 3). It is possible that as energy costs required for salt adaptation mechanisms increased, less substrate could be allocated to the synthesis of biomass. Despite differences in the fungal and bacterial contribution between salt treatments (see section 4.3.), respiration was not related to changes in the relative importance of fungi and bacteria, but rather to salinity directly (Fig. 4). This points towards a high degree of functional redundancy within the microbial community.

4.3. Bacterial and fungal growth responses to salt addition

We expected both bacterial and fungal growth to decrease with increasing salinity, as a consequence of the inhibiting effect of salinity on growth. However, our results showed a more complicated pattern of growth response to salinity that did not confirm our hypothesis (Fig. 3, 4). Bacterial growth was highest in the 7 mg NaCl g^{-1} treatment and lowest in the 22 mg NaCl g^{-1} treatment, and vice versa for fungal growth (Fig. 3, 4). There was a negative relationship between fungal and bacterial growth, likely reflecting a competitive interaction between bacteria and fungi. During decomposition fungi and bacteria compete for substrate and it has been reported that the presence of bacteria has an antagonistic effect on fungi (Mille-Lindblom et al., 2006; Rousk et al., 2008). As a result suppression of bacterial growth in the 22 mg NaCl g^{-1} by salinity could have resulted in a promotion of fungal growth, as fungi are released from competition with bacteria.

In contrast, in the 7 mg NaCl g^{-1} fungal growth likely was suppressed by high bacterial growth.

The fact that fungi could grow in the 22 mg NaCl g^{-1} treatment where bacterial growth remained low throughout the experiment (Fig. 3, 4) corroborates our hypothesis that fungi are more salt-tolerant than bacteria. This is in accordance with previous results finding less inhibition of fungal growth compared to bacterial growth in response to salt exposure (Rath et al., 2016), as well as studies that found an increase in the fungal/bacterial ratio of biomass in saline soils (Wichern et al., 2006; Kamble et al., 2014). The chitinous cell walls of fungi provides higher protection against water loss caused by low soil moisture (Lennon et al., 2012; Manzoni et al., 2012) and could conceivably also contribute to higher resistance of fungi to low water potential caused by high solute concentrations (Griffiths et al., 1998; Reischke et al., 2014). Increased fungal resistance has also been found in response to high heavy-metal exposure (Rajapaksha et al., 2004) and low pH (Rousk et al., 2010) in soils, two disturbances that also result in high extracellular cation concentrations. The interpretation is complicated by the fact that in the 7 mg NaCl g^{-1} soil bacterial growth clearly dominated (Fig. 3, 4). Possibly fast growing opportunistic bacteria took over in that treatment after severe disturbance of the community present in the soil before the addition of salt, and consequently suppressed the initially high fungal growth. In the 0 and 2 mg NaCl g^{-1} fungal and bacterial growth were both at intermediate levels (Fig. 3, 4), since salt exposure did not the lead to disruption of either group, and consequently did not result in the competitive release of fungi or bacteria.

We combined two different plant litters to equally stimulate both bacterial and fungal growth, as alfalfa promotes bacterial growth, while wheat straw promotes fungal growth (Rousk and Bååth, 2007; Grosso et al., 2016). In those treatments in which bacteria could establish, bacterial growth increased more rapidly in response to plant material addition than fungal growth (Fig. 4), which could reflect the different ecological strategies of bacteria and fungi. Bacteria are commonly associated with an r-selected strategy and fungi with a K-selected strategy for resource use (Wardle et al., 2004; de Boer et al., 2005; Laliberte et al., 2017). Due to higher growth rates on new resources bacteria were thus faster to respond after substrate addition.

5. Conclusion

We were able to induce bacterial community tolerance to salt in a non-saline soil with no history of salt exposure. Community salt tolerance induced by salt exposure developed within a time frame of ca. 1 week, indicating that even bacterial communities that are not pre-adapted can adjust rapidly to new environmental conditions. The final tolerance was linked to changes in community

composition. These findings suggest that bacterial communities are able to quickly adapt to fluctuating salt concentrations in the soil. The emergence of community salt tolerance was accompanied by recovery of initially reduced respiration and growth rates in soils receiving > 7 mg NaCl g^{-1}. Respiration increased more quickly after the addition of NaCl and plant litter than growth, which would have resulted in low substrate use efficiency following the change in salinity. We observed strong signs of competitive interactions of fungi and bacteria during decomposition of plant litter, as fungi grew better when bacterial growth was reduced by salinity, but were suppressed in treatments with high bacterial growth. These competitive interactions strongly modified the bacterial and fungal growth response to salinity. While fungi could resist higher salt concentrations than bacteria, the actual fungal and bacterial contribution to communities in saline soils was a function of both abiotic and biotic factors.

Acknowledgements

References

Bååth, E., 1994. Thymidine and leucine incorporation in soil bacteria with different cell size. Microbial Ecology 27, 267-278.

Bååth, E., 2001. Estimation of fungal growth rates in soil using C-14-acetate incorporation into ergosterol. Soil Biology & Biochemistry 33, 2011-2018.

Bååth, E., Pettersson, M., Söderberg, K.H., 2001. Adaptation of a rapid and economical microcentrifugation method to measure thymidine and leucine incorporation by soil bacteria. Soil Biology & Biochemistry 33, 1571-1574.

Berga, M., Zha, Y.H., Szekely, A.J., Langenheder, S., 2017. Functional and Compositional Stability of Bacterial Metacommunities in Response to Salinity Changes. Frontiers in Microbiology 8.

Chowdhury, N., Marschner, P., Burns, R.G., 2011. Soil microbial activity and community composition: Impact of changes in matric and osmotic potential. Soil Biology & Biochemistry 43, 1229-1236.

de Boer, W., Folman, L.B., Summerbell, R.C., Boddy, L., 2005. Living in a fungal world: impact of fungi on soil bacterial niche development. FEMS microbiology reviews 29, 795-811.

Edgar, R.C., 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nature Methods 10, 996-+.

FAO, 2011. The state of the world's land and water resources for food and agriculture : managing systems at risk, 1st ed. Earthscan, Milton Park, Abingdon ; New York, NY.

Griffiths, B.S., Ritz, K., Ebblewhite, N., Dobson, G., 1998. Soil microbial community structure: Effects of substrate loading rates. Soil Biology and Biochemistry 31, 145-153.

Grosso, F., Bååth, E., De Nicola, F., 2016. Bacterial and fungal growth on different plant litter in Mediterranean soils: Effects of C/N ratio and soil pH. Applied Soil Ecology 108, 1-7.

Hasbullah, H., Marschner, P., 2015. Residue properties influence the impact of salinity on soil respiration. Biology and Fertility of Soils 51, 99-111.

Horikoshi, K.k., Antranikian, G., Bull, A.T., Robb, F.T., Stetter, K.O., 2011. Extremphiles Handbook. Springer Japan.

James, F.C., Mcculloch, C.E., 1990. Multivariate-Analysis in Ecology and Systematics - Panacea or Pandora Box. Annual Review of Ecology and Systematics 21, 129-166.

Jobbagy, E.G., Jackson, R.B., 2004. Groundwater use and salinization with grassland afforestation. Global Change Biology 10, 1299-1312.

Kakumanu, M.L., Williams, M.A., 2014. Osmolyte dynamics and microbial communities vary in response to osmotic more than matric water deficit gradients in two soils. Soil Biology and Biochemistry 79, 14-24.

Kamble, P.N., Gaikwad, V.B., Kuchekar, S.R., Bååth, E., 2014. Microbial growth, biomass, community structure and nutrient limitation in high pH and salinity soils from Pravaranagar (India). European Journal of Soil Biology 65, 87-95.

Laliberte, E., Kardol, P., Didham, R.K., Teste, F.P., Turner, B.L., Wardle, D.A., 2017. Soil fertility shapes belowground food webs across a regional climate gradient. Ecology Letters 20, 1273-1284.

Lennon, J.T., Aanderud, Z.T., Lehmkuhl, B.K., Schoolmaster, D.R., 2012. Mapping the niche space of soil microorganisms using taxonomy and traits. Ecology 93, 1867-1879.

Manzoni, S., Schimel, J.P., Porporato, A., 2012. Responses of soil microbial communities to water stress: results from a meta-analysis. Ecology 93, 930-938.

McDonald, D., Price, M.N., Goodrich, J., Nawrocki, E.P., DeSantis, T.Z., Probst, A., Andersen, G.L., Knight, R., Hugenholtz, P., 2012. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. Isme Journal 6, 610-618.

Mille-Lindblom, C., Fischer, H., J. Tranvik, L., 2006. Antagonism between bacteria and fungi: substrate competition and a possible tradeoff between fungal growth and tolerance towards bacteria. Oikos 113, 233-242.

Mouginot, C., Kawamura, R., Matulich, K.L., Berlemont, R., Allison, S.D., Amend, A.S., Martiny, A.C., 2014. Elemental stoichiometry of Fungi and Bacteria strains from grassland leaf litter. Soil Biology & Biochemistry 76, 278-285.

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2016. vegan: Community Ecology Package, R package version 2.4-1.

Oren, A., 1999. Bioenergetic aspects of halophilism. Microbiology and Molecular Biology Reviews 63, 334-+.

Pankhurst, C.E., Yu, S., Hawke, B.G., Harch, B.D., 2001. Capacity of fatty acid profiles and substrate utilization patterns to describe differences in soil microbial communities associated with increased salinity or alkalinity at three locations in South Australia. Biology and Fertility of Soils 33, 204-217.

Rajapaksha, R.M.C.P., Tobor-Kaplon, M.A., Baath, E., 2004. Metal toxicity affects fungal and bacterial activities in soil differently. Applied and Environmental Microbiology 70, 2966-2973.

Ramirez, K.S., Leff, J.W., Barberan, A., Bates, S.T., Betley, J., Crowther, T.W., Kelly, E.F., Oldfield, E.E., Shaw, E.A., Steenbock, C., Bradford, M.A., Wall, D.H., Fierer, N., 2014. Biogeographic patterns in below-ground diversity in New York City's Central Park are similar to those observed globally. Proceedings of the Royal Society B-Biological Sciences 281.

Rath, K.M., Maheshwari, A., Bengtson, P., Rousk, J., 2016. Comparative toxicity of salts to microbial processes in soil. Applied and Environmental Microbiology, AEM. 04052-04015.

Rath, K.M., Rousk, J., 2015. Salt effects on the soil microbial decomposer community and their role in organic carbon cycling: A review. Soil Biology & Biochemistry 81, 108-123.

Reischke, S., Rousk, J., Bååth, E., 2014. The effects of glucose loading rates on bacterial and fungal growth in soil. Soil Biology and Biochemistry 70, 88-95.

Rengasamy, P., 2006. World salinization with emphasis on Australia. Journal of Experimental Botany 57, 1017-1023.

Rousk, J., Bååth, E., 2007. Fungal and bacterial growth in soil with plant materials of different C/N ratios. Fems Microbiology Ecology 62, 258-267.

Rousk, J., Brookes, P.C., Bååth, E., 2010. Investigating the mechanisms for the opposing pH relationships of fungal and bacterial growth in soil. Soil Biology & Biochemistry 42, 926-934.

Rousk, J., Demoling, L.A., Bahr, A., Bååth, E., 2008. Examining the fungal and bacterial niche overlap using selective inhibitors in soil. Fems Microbiology Ecology 63, 350-358.

Rousk, J., Elyaagubi, F.K., Jones, D.L., Godbold, D.L., 2011. Bacterial salt tolerance is unrelated to soil salinity across an arid agroecosystem salinity gradient. Soil Biology & Biochemistry 43, 1881-1887.

Sardinha, M., Muller, T., Schmeisky, H., Joergensen, R.G., 2003. Microbial performance in soils along a salinity gradient under acidic conditions. Applied Soil Ecology 23, 237-244.

Schmidt, M.W.I., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I.A., Kleber, M., Kogel-Knabner, I., Lehmann, J., Manning, D.A.C., Nannipieri, P., Rasse,

D.P., Weiner, S., Trumbore, S.E., 2011. Persistence of soil organic matter as an ecosystem property. Nature 478, 49-56.

Setia, R., Marschner, P., Baldock, J., Chittleborough, D., Smith, P., Smith, J., 2011. Salinity effects on carbon mineralization in soils of varying texture. Soil Biology & Biochemistry 43, 1908-1916.

Shade, A., Peter, H., Allison, S.D., Baho, D.L., Berga, M., Burgmann, H., Huber, D.H., Langenheder, S., Lennon, J.T., Martiny, J.B.H., Matulich, K.L., Schmidt, T.M., Handelsman, J., 2012. Fundamentals of microbial community resistance and resilience. Frontiers in Microbiology 3.

Six, J., Frey, S.D., Thiet, R.K., Batten, K.M., 2006. Bacterial and fungal contributions to carbon sequestration in agroecosystems. Soil Science Society of America Journal 70, 555-569.

Strickland, M.S., Rousk, J., 2010. Considering fungal:bacterial dominance in soils - Methods, controls, and ecosystem implications. Soil Biology & Biochemistry 42, 1385-1395.

Tripathi, S., Kumari, S., Chakraborty, A., Gupta, A., Chakrabarti, K., Bandyapadhyay, B.K., 2006. Microbial biomass and its activities in salt-affected coastal soils. Biology and Fertility of Soils 42, 273-277.

Turk, M., Montiel, V., Žigon, D., Plemenitaš, A., Ramos, J., 2007. Plasma membrane composition of Debaryomyces hansenii adapts to changes in pH and external salinity. Microbiology 153, 3586-3592.

Vass, M., Langenheder, S., 2017. The legacy of the past: effects of historical processes on microbial metacommunities. Aquatic Microbial Ecology 79, 13-19.

Wardle, D.A., Walker, L.R., Bardgett, R.D., 2004. Ecosystem properties and forest decline in contrasting long-term chronosequences. Science 305, 509-513.

Wichern, J., Wichern, F., Joergensen, R.G., 2006. Impact of salinity on soil microbial communities and the decomposition of maize in acidic soils. Geoderma 137, 100-108.

Zhang, L., Dawes, W.R., Slavich, P.G., Meyer, W.S., Thorburn, P.J., Smith, D.J., Walker, G.R., 1999. Growth and ground water uptake responses of lucerne to changes in groundwater levels and salinity: lysimeter, isotope and modelling studies. Agricultural Water Management 39, 265-282.

Zhang, Y.-M., Rock, C.O., 2008. Membrane lipid homeostasis in bacteria. Nature Reviews Microbiology 6, 222.

Supplemental material

Table S1:

Table of abundant OTUs (>0.5% of reads) that were positively correlated (Spearman's rank correlation coefficient ρ >0.5) with bacterial community salt tolerance.

OTU ID	ρ[1]	Maximum abundance (% reads)[2]	Taxonomy
OTU_1219	0.94	0.96	k__Bacteria; p__Firmicutes; c__Bacilli; o__Bacillales; f__; g__; s__
OTU_47	0.80	1.5	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhizobiales; f__Phyllobacteriaceae; g__Aminobacter; s__
OTU_31	0.80	24	k__Bacteria; p__Firmicutes; c__Bacilli; o__Bacillales; f__Sporolactobacillaceae; g__; s__
OTU_6238	0.80	14	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Xanthomonadales; f__Xanthomonadaceae; unclassified; unclassified
OTU_229	0.79	16	k__Bacteria; p__Firmicutes; c__Bacilli; o__Bacillales; f__Bacillaceae; unclassified; unclassified
OTU_342	0.79	10	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Brevibacteriaceae; g__Brevibacterium; s__aureum
OTU_1140	0.79	0.95	k__Bacteria; p__Firmicutes; c__Bacilli; o__Bacillales; f__Paenibacillaceae; unclassified; unclassified
OTU_404	0.76	13	k__Bacteria; p__Firmicutes; c__Bacilli; o__Bacillales; f__Bacillaceae; g__Gracilibacillus; s__dipsosauri
OTU_298	0.76	12	k__Bacteria; p__Firmicutes; c__Bacilli; o__Bacillales; f__Bacillaceae; g__Pontibacillus; s__
OTU_893	0.76	2.3	k__Bacteria; p__Firmicutes; c__Bacilli; o__Bacillales; unclassified; unclassified; unclassified
OTU_1650	0.76	1.3	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; f__Dermabacteraceae; g__Brachybacterium; s__
OTU_1837	0.76	0.69	k__Bacteria; p__Firmicutes; c__Bacilli; o__Bacillales; unclassified; unclassified; unclassified
OTU_545	0.73	4.5	k__Bacteria; p__Firmicutes; c__Bacilli; o__Bacillales; unclassified; unclassified; unclassified
OTU_293	0.66	10	k__Bacteria; p__Bacteroidetes; c__[Saprospirae]; o__[Saprospirales]; f__Chitinophagaceae; g__; s__
OTU_300	0.60	1.5	k__Bacteria; p__Verrucomicrobia; c__[Spartobacteria]; o__[Chthoniobacterales]; f__[Chthoniobacteraceae]; g__DA101; s__
OTU_7650	0.59	6.1	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Xanthomonadales; f__Xanthomonadaceae; g__Rhodanobacter; s__lindaniclasticus
OTU_3354	0.52	2.9	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhizobiales; unclassified; unclassified; unclassified

[1] Spearman's rank correlation coefficient

[2] Maximum proportion of reads in one sample.

[3] Taxonomy assigned to the OTU sequence: k= kingdom, p= phylum, c=class, o=order, f= family, g=genus, s=species

Using a trait-based approach to link bacterial community composition to salinity along soil salinity gradients

Kristin M. Rath[1,2], Noah Fierer[3], Daniel Murphy[4] and Johannes Rousk[1]

[1] Section of Microbial Ecology, Dept. of Biology, Lund University, Lund, Sweden

[2] Centre for Environmental and Climate Research (CEC), Lund University, Lund, Sweden

[3] Cooperative Institute for Research in Environmental Sciences, University of Colorado, Boulder, CO, USA

[4] School of Agriculture and Environment, The University of Western Australia, Crawley, WA, Australia

Abstract

A central question in the field of microbial ecology is to predict responses of microbial communities to environmental change. Salinity has been established as one of the most important factors driving the composition of microbial communities in aquatic systems, but despite large spans of salinities held in soils its role in shaping soil communities is underexplored. A drawback of exploratory studies on environmental drivers of community composition is the difficulty of linking shifts in composition causally to one factor. Here, we use a trait-based approach to identify direct effects of salinity on soil bacterial communities along two salinity gradients. Through dose-response relationships between salinity and bacterial growth we effectively quantified distributions of the trait salt tolerance on the community level. Community salt tolerance was closely correlated to the soil environment, indicating a strong filtering effect of salinity on the communities. At high salinities, bacterial community compositions along the two gradients converged. A number of taxa could be identified that showed a consistent positive response to increasing salinity and could be used as potential biomarkers for high community salt tolerance. Through increased community salt tolerance the loss of microbial functioning associated with high salinity was reduced in saline soils compared to the effect of salinity in non-saline soils. By quantifying the distribution of the functional trait salt tolerance at the community level we could identify salinity as a strong driver of microbial community composition in soils.

Introduction

Soils harbor an enormous diversity of microorganisms, which are responsible for carrying out many important processes related to the decomposition of organic matter and nutrient cycling. One of the major challenges in the field of microbial ecology is to move beyond descriptive reports of patterns in community composition and find a basis for predicting shifts in bacterial communities in response to environmental change (Antwis et al., 2017). Unfortunately, our understanding of the underlying dynamics structuring bacterial communities remains limited. Differences in community composition have primarily been linked to edaphic factors based on correlative relationships (Lauber et al., 2008). In particular, a large proportion of the beta diversity in soil bacterial communities is correlated with differences in soil pH (Lauber et al., 2009; Rousk et al., 2010). In a global survey of bacterial communities from both terrestrial and aquatic environments, salinity emerged as the dominant factor linked to bacterial community composition (Lozupone and Knight, 2007) and has been reported to be the factor most strongly correlated with community composition in aquatic systems (Herlemann et al., 2011; Logares et al., 2013).

Saline soils, commonly defined as soils with an electrical conductivity (EC) in a saturated soil extract of more than 4 dS m^{-1} (Richards, 1954), cover a large area in arid and semi-arid regions of the globe. Globally, it is estimated that around 900 million hectares of soil are affected to some degree by high ion concentrations (Szabolcs, 1989). ECs in saline soils can reach more than 200 dS m^{-1} (Pandit et al., 2015), matching the EC of the saltiest bodies of water. Considering that soil salinities cover ranges spanning several orders of magnitude of EC (<0.1 dS m^{-1} in non-saline soils to >100 dS m^{-1} in highly saline soil) it should be expected that salinity has a similarly strong effect on bacterial community composition in soil as has been observed in aquatic systems, and that soil salinity could be another strong driver of soil microbial community composition in addition to soil pH. Studies on the composition of bacterial communities of saline soils found shifts in community composition associated with salinity, as well as a decline in phylogenetic diversity as salinity increased (Hollister et al., 2010; Canfora et al., 2014; Van Horn et al., 2014; Xie et al., 2017). However, general principles that could ultimately lead to a predictive understanding of the microbial community response to soil salinity have yet to emerge.

When identifying environmental drivers of bacteria community composition based on correlative relationships, the influence of confounding factors is difficult to tease apart from the direct impact of the environmental factor of interest. For instance, soil salinity is frequently correlated with reduced organic matter input (Wong et al., 2008; Setia et al., 2013), and soil pH (Rengasamy, 2010). To isolate direct effects of environmental factors on the community, trait-based approaches

have been proposed (Webb et al., 2010; Wallenstein and Hall, 2012). However, information on functional traits on the level of single bacterial taxa is largely unavailable (Green et al., 2008), Measuring traits aggregated at the community level can be used as an alternative to measure the distribution of a trait within the community (Wallenstein and Hall, 2012; Fierer et al., 2014). This approach has been used to study microbial trait distributions including those of pH tolerance (Bååth and Kritzberg, 2015; Bárcenas-Moreno et al., 2016), salt tolerance (Rousk et al., 2011; Kamble et al., 2014) and heavy metal tolerance (Fernández-Calviño et al., 2012) among others. While tolerance to environmental factors has been studied both on the level of communities, as well as on the level of cultured species, these tolerance trait surveys have rarely been linked to community composition.

Bacterial activity and functioning are inhibited by salinity through both osmotic pressure and specific ion toxicity in response to short-term salt exposure (Rath et al., 2016). Salt-tolerant microorganisms have developed a number of physiological adaptations and mechanisms to withstand high concentrations of salt in their environment (Oren, 1999), which could allow communities with high relative abundances of salt-tolerant members to overcome salt-induced limitations on bacterial functioning. However, microbial processes related to the decomposition of organic material are usually reduced in naturally saline soils as well, where communities would presumably have adapted to the local environmental conditions (Rath and Rousk, 2015). Partially this could be an effect of low organic matter quantity and quality in saline soils (Setia et al., 2013), so supplying additional organic matter to saline soils could alleviate the inhibiting effect of salinity on microbial functioning.

In this study we tested the connection between community composition and the distribution of the trait salt tolerance along gradients of soil salinity. We used two salinity gradients, of which one was confounded by a pH gradient. To distinguish the effects of salinity from those of pH, we also established pH optima for the communities, to see where along the gradient pH had an impact on constraining community composition. Along the gradients we expected that (i) the local environment would have selected for a community with trait distributions for salt tolerance and pH tolerance that are matched to the environment, (ii) that differences in salt tolerance and pH tolerance would be reflected in differences in the community composition, (iii) that differences in the community composition with increasing salt tolerance would be consistent across both gradients and (iv) that increased community salt tolerance in high salinity environments would offset salt-inhibition of microbial process rates and would enable microbial functioning to continue at higher rates than predicted from process rate inhibition in short-term exposure to salt.

Material and methods

Sites

Samples were collected from two salt gradients located along Lake O'Connor in Western Australia that differed in land use at the less saline sites. One gradient was located on the northern shore of the lake (henceforth the agricultural [AG] gradient) (32° 28'S, 119°12'E), the other gradient on the southern shore of the lake (henceforth the natural vegetation [NV] gradient) (32°29'S, 119°13'E) (Fig. S1). The distance between the two gradients was ca. 2 km. Each gradient was sampled in 3 transects of 8 sampling points, resulting in a total of 24 samples collected for each gradient. Along the gradients, vegetation changed with distance from the lakeshore (SI Appendix). Electrical conductivity, pH and organic matter contents of samples were determined using standard methods (SI Appendix). The amount of water-soluble cations (Ca^{2+}, K^+, Mg^{2+}, Na^+) was analyzed in water extractions from soil with ICP-OES (Optima 8300, Perkin Elmer).

Determination of trait distributions of salt and pH tolerance

Prior to measuring community salt and pH tolerance, soils were incubated together with 5 mg g^{-1} alfalfa-straw mixture for one month at room temperature to boost bacterial growth rates. After this pre-incubation period assays to determine the trait distributions of salt and pH tolerance were performed. Soil suspensions were created by mixing 1 g of soil with 20 ml of water. After homogenization and centrifugation (1000 g), aliquots (1.35 ml) of bacterial suspension were transferred to 2 ml microcentrifugation tubes and were then adjusted to either different electrical conductivities (suspension EC) or different pH-values (suspension pH).

To change suspension EC, the soil suspensions were mixed with a range of NaCl additions to create a gradient of 8 different salt concentrations (including one control level without salt addition) in a final volume of 1.5 ml. Target concentrations of NaCl additions ranged from 5.5 mol/l to 0.007 mol/l, with the achieved suspension EC depending on the initial EC of the soil suspension. To adjust suspension pH, suspensions were mixed with 0.15 ml of a citrate-phosphate pH buffer (11 different levels ranging from pH 3.0 to 8.0) or distilled H_2O, resulting in a range of 12 different pHs for each sample. The suspension pH was validated with a pH meter. Following the adjustment of salinity or pH in the soil suspensions bacterial growth was measured (SI Appendix).

Bacterial community composition and functioning

For each sample, the maximal bacterial growth rate measured in the salt tolerance assay was used to compare bacterial growth between samples. Respiration was

measured in headspace vials using a gas chromatograph (GC). Microbial biomass was determined through substrate-induced respiration (SIR) (SI Appendix). DNA was extracted using the MoBio PowerSoil DNA Isolation Kit (Carlsbad, CA, USA) according to the manufacturer's recommendations. Extracted DNA was amplified using primers targeting the V4-V5 region of the bacterial 16S rRNA gene. Sequencing and preparation of sequencing data was carried out as described in Oliverio et al. (2017). Before downstream analyses samples were rarefied to 10 000 reads and samples containing less than 10 000 reads were removed from further analysis (for details see SI Appendix).
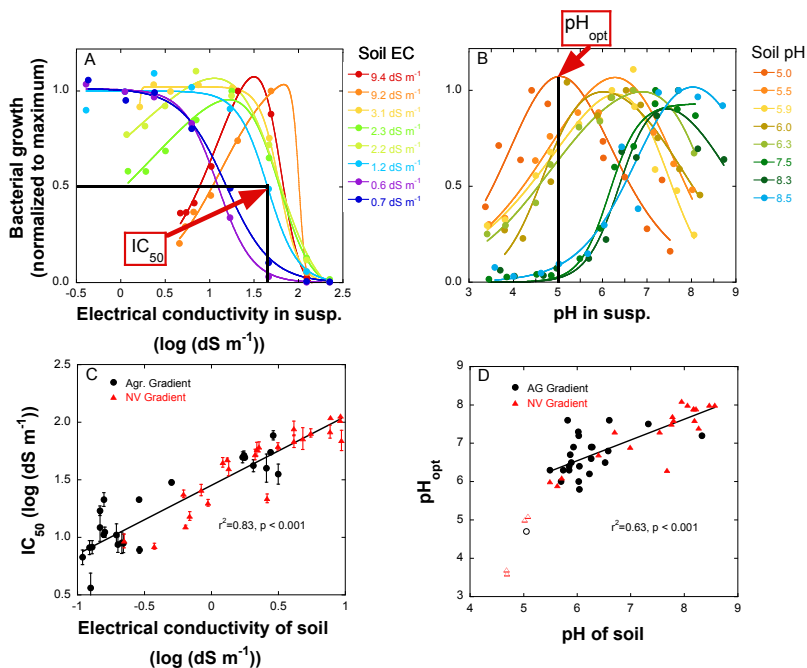

Fig. 1
Response curves between bacterial growth and (A) suspension electrical conductivity (EC) and (B) suspension pH. Depicted are representative examples of response curves of soils of different soil EC and soil pH. Values of soil EC (A) and soil pH (B) measured in a 1:5 soil:water mixture of the selected soils are given in the figure legends. Bacterial growth was normalized to the maximum growth rate in each sample. The relationships between bacterial growth and EC and pH were described with logistic or double logistic functions. To describe the community-level salt tolerance in a sample, the IC_{50} value (suspension EC at which bacterial growth was inhibited by 50% compared to the maximum) was used. To describe the community-level pH tolerance the suspension pH at which growth reached its maximum was used (pH_{opt}). (C) Relationship between soil EC and community-level salt tolerance (indicated by IC_{50}) along both salinity gradients. (D) Relationship between soil pH and community-level pH tolerance (indicated by pH_{opt}). Samples with a pH <5.5 (open symbols) were excluded from the regression analysis.

Data analysis

Growth rates in the salt and pH tolerance assays were normalized to growth rates measured at the optimum EC or pH for each sample. Dose-response relationships between bacterial growth and suspension EC or pH were established using logistic models (SI Appendix). Community-level distribution of salt tolerance was quantified using the suspension EC at which bacterial growth is inhibited by 50% (IC_{50}) with increasing salinity. The suspension pH at which bacterial growth reached its optimum (pH_{opt}) in each sample was used as an indicator of the community-level trait distribution of pH tolerance. Linear regression models were used to test for significant correlations between community-level trait indicators (IC_{50} and pH_{opt}) and soil EC and pH. Analysis of the link between bacterial community composition and functional trait distributions was carried out using multivariate statistics (SI Appendix). Multiple linear regression models were used to test for a significant correlation between environmental variables and diversity, as well as functional parameters (SI Appendix).

Results

Characterization of gradients

The AG gradient encompassed soil ECs ranging from 0.1 dS m^{-1} to 3 dS m^{-1} measured in a 1:5 soil:water mixture, while the soil pH along the gradient was between 5.5 and 7.0 (Fig. S2). The NV gradient encompassed soil ECs ranging from 0.2 dS m^{-1} to 9 dS m^{-1}. Soil pH along the gradient ranged from ca. 4.5 to ca. 8.5. Along the NV gradient, a significant negative linear correlation was found between soil EC and soil pH ($R^2 = 0.50$, p<0.001), whereas along the AG gradient soil EC and soil pH were not significantly correlated (Fig. S2). OM content along the AG gradient ranged from 2.8 to 4.8% dry weight (dw) and was positively correlated with soil pH ($R^2=0.26$, p<0.01), but not with soil EC (Fig. S3). Along the NV gradient OM content ranged from 7.6 to 10.0% dw, and was neither correlated with soil EC nor soil pH (Fig. S3). Na$^+$ was the predominant cation in sites from both gradients (Fig. S4).

Community trait distributions

In samples from sites with lower salinity, bacterial growth was inhibited with increasing salinity (Fig. 1A). The sigmoidal relationship between growth and the logarithm of the suspension EC could be modeled with a logistic function (R^2 from 0.95 to 0.99). In communities from high salinity samples bacterial growth was inhibited by both low and high suspension EC (Fig. 1A). For these samples, a double logistic function could fit the relationships between suspension EC and bacterial growth well (R^2 from 0.91 to 0.99). In the most saline samples the

suspension EC at which communities had their growth optimum was about a factor of 10 higher than that measured in a 1:5 soil:water suspension.

We used the $\log IC_{50}$ as an indicator of the community-level distribution of the trait of salt tolerance. There was a strong positive relationship between the logarithm of the soil EC and the $\log IC_{50}$ (R^2=0.83, p<0.001) (Fig 1C). The difference between soil EC and IC_{50} decreased with increasing salinity. In the most saline samples the IC_{50} was about 10 times higher than the soil EC, whereas in the least saline samples IC_{50} was 70 times higher than soil EC.

The relationships between bacterial growth and suspension pH could also be modeled with a double-logistic function (R^2 from 0.46 to 0.99, with a mean R^2=0.91) (Fig. 1B). The indicator used for the distribution of the trait pH tolerance was the pH_{opt} for bacterial growth. Above soil pH 5.5 there was a good linear relationship between soil pH and pH_{opt} (R^2=0.63, p<0.001) (Fig. 1D). For every 1 unit increase in soil pH , the optimum pH increased by ca. 0.5 units. Consequently, around soil pH 5.5 pH_{opt} was around 0.8 units higher than the soil pH, whereas around soil pH 8.5 pH_{opt} was around 0.6 units lower than the soil pH.
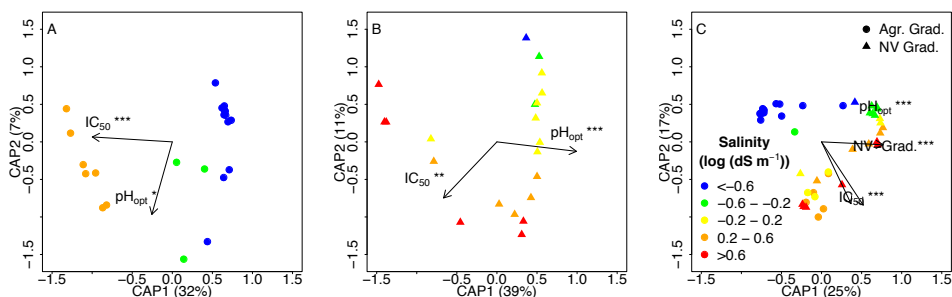

Fig. 2
Canonical Analysis of Principal Coordinates (CAPSCALE) derived from Bray-Curtis dissimilarities of the community composition of sampling points based on 16S rRNA gene amplicon sequencing. (A) samples from the AG Gradient, (B) samples from the NV Gradient, (C) samples from both gradients. Numbers in parentheses in the axis labels give the percentage of variance accounted for by the principal coordinates. Community salt tolerance (IC_{50}), community pH tolerance (pH_{opt}) and a dummy variable for gradient were used as constraining variables. The significance of constraining variables was tested with PERMANOVA.

Community composition

In total, 3035 OTUs occurred at least 10 times in the dataset. In the samples from the AG gradient we found 2020 different OTUs and in the NV gradient 1897 different OTUs. Of these OTUs, 882 were found in samples from both gradients, whereas the rest were unique to either gradient. 97% of reads belonged to

Bacteria, and 3% were assigned to Archaea. The phylum Proteobacteria made up 41% of reads, followed by Bacteroidetes (15%), Actinobacteria (14%), Gemmatimonadetes (6%) and Planctomycetes (5%). The most abundant family were the Sphingomonadaceae, a family of Alphaproteobacteria, which accounted for 7% of all reads. Other families that made up >3% of reads were the Chitinophagaceae (Bacteroidetes) and the Xanthomonadaceae (Gammaproteobacteria).

Community composition changed in a similar direction with increasing salt tolerance along both gradients (Fig. 2). The distance between samples from different gradients was larger at low salinity sites, whereas at high salinity sites, samples from different gradients became more similar (Fig. 2C). Both salt and pH tolerance explained significant amounts of community composition, with a higher proportion of variation being related to salt tolerance (Fig. 2). Overall community composition along both gradients was significantly correlated with salt tolerance (Mantel test; r=0.35, p<0.001) and pH tolerance (Mantel test; r=0.56, p<0.001), with shifts in community composition being better correlated with changes in the trait distribution of pH tolerance than salt tolerance (Fig. S7 A,B). Along the AG gradient, community composition was more strongly correlated with salt tolerance (Mantel test; r=0.54, p<0.001) than pH tolerance (Mantel test; r=0.34, p<0.01) (Fig. S7 C,D), whereas along the NV gradient, the correlation between community composition and salt tolerance (Mantel test; r=0.29, p<0.01) was weaker than that with pH tolerance (Mantel test; r=0.84, p<0.001) (Fig. S7 E,F).

Overall, an increase in pH tolerance by 1 pH unit was connected to a shift in community composition of a similar magnitude as the change in community composition associated with an increase of $logIC_{50}$ by 0.5 log (dS m^{-1}), i.e. a 3.5-fold increase in salt tolerance (Fig. S7 A,B), with marginal differences between gradients (Fig. S7 C-F). Changes in salt tolerance were connected to greater shifts in the community composition along the AG gradient than along the NV gradient. An increase in the salt tolerance by 1 log (dS m^{-1}) (i.e. a 10-fold increase) along the AG gradient (Fig. S7 C) was connected to a community shift of the same magnitude as an increase by ca. 1.7 log (dS m^{-1}) (i.e. 50-fold increase) along the NV gradient (Fig. S7 E).

In the AG gradient 46 OTUs with a relative abundance ≥ 1% were found to be positively correlated with salt tolerance (Table S1). The OTU with the strongest positive correlation was an unclassified Gammaproteobacterium, followed by another Gammaproteobacterium of the genus *Salinisphaera* and a member of the Gemmatimonadetes group 5. 15 of the 46 positively correlated OTUs belonged to the Gammaproteobacteria, making it the taxonomic group most strongly associated with increased community-level salt tolerance.

In the NV gradient, 19 OTUs were positively correlated with salt tolerance, of which 10 were also found to be positively correlated with salt tolerance in the AG gradient (Table S1). Of these 10 OTUs, 9 were only observed in communities with an IC_{50} above 1.5 log (dS m^{-1}). The class Gammaproteobacteria made up 10 of the positively correlated OTUs in the NV gradient. The OTUs most strongly associated with salt tolerance included the same strain of unclassified Gammaproteobacteria and *Salinisphaera* that had the strongest correlation with salt tolerance in the AG gradient.

Diversity

Shannon diversity declined along the gradients with increasing salinity and decreasing pH (Fig. S7). The multiple linear regression models for Shannon diversity were significant for both the AG gradient ($F_{(2,20)}$ = 14.9, R^2=0.56, p<0.001) and the NV gradient ($F_{(2, 18)}$ = 49.9, R^2=0.83, p<0.001). In the AG gradient both logEC (p<0.001) and pH (p<0.05) significantly predicted Shannon diversity. In the NV gradient, only pH was found to significantly predict Shannon diversity (p<0.001), whereas logEC did not predict a significant proportion of the variation in diversity beyond variation also attributed to pH.
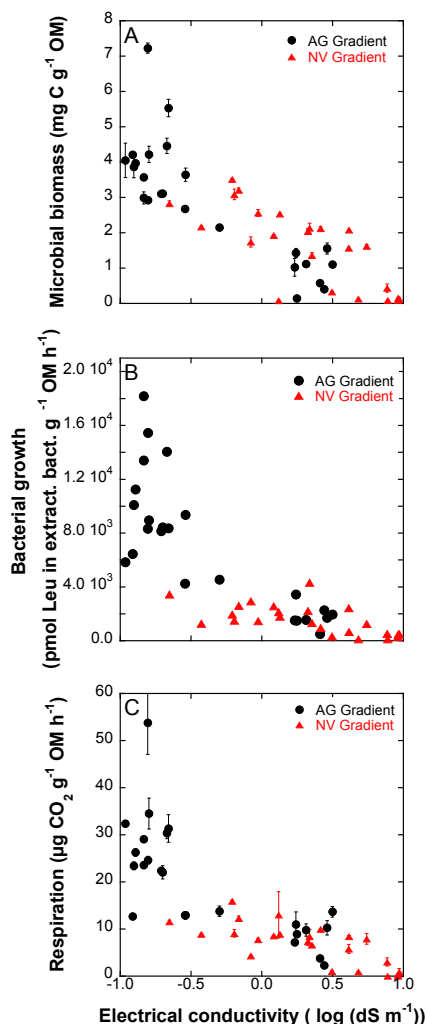
Fig. 3

(A) Microbial biomass derived substrate induced respiration (SIR), (B) bacterial growth measured as incorporation of ^{3}H-labelled leucine into bacterial biomass, and (C) respiration along the salinity gradients after one month of incubation with 5 mg g^{-1} soil 1:1 alfalfa-wheat straw mixture.

Microbial biomass, growth and respiration

Microbial biomass, bacterial growth and respiration all declined with increasing salinity (Fig. 3). Microbial biomass was significantly correlated with both logEC ($p < 0.001$), and pH ($p < 0.05$). Similarly to biomass, bacterial growth rates and respiration were significantly correlated with both logEC ($p < 0.001$ and $p < 0.001$) and pH ($p < 0.01$ and $p < 0.05$). In both gradients bacterial growth was

inhibited by ca. 80% at the saline end of the gradient compared to the least saline samples in the gradient (Fig. 3B). Respiration in the most saline samples was inhibited by ca. 60% in the AG gradient and ca. 90% in the NV gradient. In the NV gradient, both bacterial growth and respiration were maintained at a constant level until a soil salinity of ca. 3 dS m^{-1}.

Discussion

Trait-environment relationships

In this study we measured the expression of tolerance traits at a functional level to infer the underlying distribution within the community and used the established trait-environment relationships to connect an environmental factor to community composition. Salinity is a strong candidate as an important driver of microbial community composition in soils, based on its strong correlation with community composition in aquatic systems (Lozupone and Knight, 2007). Our approach to estimate community tolerance to salt with dose-response relationships between salinity and growth worked effectively, and we observed substantial and systematic variation along the environmental gradients. The two salinity gradients cover much of the range of salinities globally observable in soils, from non-saline to hypersaline, including sites where salt concentrations in the pore water approached saturated conditions.

We hypothesized that in a community inhabiting a more saline site the local environment would have selected for higher salt tolerance, thereby increasing the amount of salinity required to inhibit growth. In accordance with our hypothesis, community-level salt tolerance increased proportionally to the increase in soil salinity (Fig. 1B). The strong correlation between the environment and bacterial salt tolerance provide evidence for a substantial filtering effect of increasing salinity on the trait distribution of salt tolerance within the communities along the gradient. These results also demonstrate that salinity posed a strong direct constraint on the microbial community, indicating that differences in community composition observed with increasing salt tolerance were caused directly by salinity. As salinity increased, differences between soil salinity and bacterial salt tolerance became smaller (Fig. 2), which could be the result of an increasing strength of salinity as a selective factor.

Along the NV gradient, soil salinity was negatively correlated with soil pH, with samples of the highest salinities having a pH below 5.5 (Fig. S2). The span in soil pH along the NV gradient, from ca. 4.5 to ca. 8.5, encompasses the range of soil pH values that are commonly found in soils around the globe. Soil pH is the factor that was found to be the most strongly correlated with microbial community composition in large-scale comparisons of soil communities (Lauber et al., 2009;

Griffiths et al., 2011). Similar to the observed increase in community salt tolerance with salinity, we expected community tolerance to pH to shift along the pH gradient. Consistent with our expectation we found a good correlation between soil pH and pH_{opt} ($R^2 = 0.63$, $p < 0.001$).

Linking functional trait distributions to community composition

The observed shifts in community salt tolerance as well as pH tolerance along the gradients (Fig. 1B, D) indicate that both environmental factors had resulted in filtered distributions for the selected traits within the communities. As such, this approach enabled us to identify direct effects of the studied environmental factors. One of the main mechanisms through which the trait distribution of tolerance in a community could change includes shifts in community composition, with better adapted species replacing less adapted ones. Both the distributions of salt tolerance and of pH tolerance were strongly correlated with community composition along the gradients (Fig. 2, S7). Along the two gradients differences in community compositions between samples could be large, with Bray-Curtis dissimilarities close to 1 between some of the samples, i.e. these samples shared almost no OTUs. At the low salinity end of the gradients, differences between samples from different gradients were larger than at the highly saline end of the gradients. Where communities were not constrained by salinity, differences in community composition between gradients would have been driven by other environmental factors, such as differences in pH, land use or vegetation. As salinity increased and started to act as a constraint on the microbial community, compositions became more similar between samples from the two gradients (Fig. 2C). Likely only a limited set of bacterial taxa could withstand the considerable stresses imposed on the bacterial community in the highly saline soils.

There are conflicting reports which phylogenetic groups would be expected to increase in response to soil salinity. High salinity is often associated with a switch towards an archaeal-dominated community (Ventosa et al., 2014; Pandit et al., 2015; Vavourakis et al., 2016; Zhong et al., 2016). However, in our study system we saw no change in the abundance of archaeal sequences along the salinity gradients and archaeal sequences never made up more than a few percent of the community. Previous studies exhibited a high degree of variability in which taxonomic groups are positively correlated to salinity (Canfora et al., 2014; Geyer et al., 2014; Van Horn et al., 2014; Morrissey and Franklin, 2015), which makes it difficult to derive meaningful predictions of bacterial community responses to increasing salinity. In addition it is unknown whether high-level taxonomic groupings such as phyla carry ecological relevance. We found high community salt tolerance to be positively correlated primarily with OTUs belonging to two high-level phylogenetic groups, namely the Bacteroidetes and the

Gammaproteobacteria, with only a few other phyla represented among the OTUs associated with high salt tolerance, indicating that the trait salt tolerance is more strongly represented in these taxonomic groups. Sequences from these groups found in high salinity sites of both gradients could be targeted for the use as biomarker sequences for salt-affected communities.

Comparing salt and pH tolerance

One of the objectives of this study was to establish the potential of salinity, the major factor correlated with shifts in community composition in aquatic systems (Lozupone and Knight, 2007; Herlemann et al., 2011), to drive bacterial community composition in terrestrial ecosystems. In our study system we could use the trait-environment relationships established between soil salinity and salt tolerance, and soil pH and pH tolerance to compare the impact of salinity in a terrestrial system to that of pH. Overall, salt tolerance could account for a higher proportion of the total variation in community composition in the total dataset (Fig. 2C). In contrast, along the NV gradient, which was confounded with pH, a larger proportion of the variation in community composition was connected to changes in pH tolerance (Fig. 2B).

Overall a 3.5-fold increase in salt tolerance was equivalent to an increase in pH tolerance by 1 pH unit (Fig. S7A, B). Along the salinity gradients, a larger shift in the community composition per unit change in salt tolerance was observed in the AG gradient (Fig S7C), which covered a narrower range of salinities than the NV gradient (Fig. S7D). It is possible that as salinity in the soil solution approached more extreme values, further increases in salinity from already saline conditions resulted in smaller changes in community composition. With salinity becoming the main factor constraining community composition, the pool of species capable of surviving these extreme conditions and thus able to replace less adapted ones would have decreased. This would imply that increases in salinity in non- or oligo-saline habitats above levels at which salinity is affecting bacterial functioning would result in larger shifts in the bacterial community composition than increases in salinity in already highly saline soils. In contrast, changes in pH tolerance were connected to shifts in community composition of similar magnitude along both gradients, despite the AG gradient covering a much shorter range of pH-values. However, pH values between ca. 5 and 8 are frequent in soils (Lauber et al., 2009; Griffiths et al., 2011). Therefore the span of soil pH values covered by the gradients arguably provided less extreme conditions for bacteria than the upper limit of the range of salinities found along the gradient.

Effect of salt tolerance on microbial functioning

To estimate the effect of salinity on bacterial performance, bacterial growth, in the form of incorporation of leucine into biomass, and respiration rates were measured. These can be considered as proxies for anabolic and catabolic processes respectively that are carried out by the bacterial community. The salinities measured in soils from the saline end of the gradients corresponded to salinities that were strongly inhibitory to bacterial growth in the salt tolerance assay to communities from non-saline or slightly saline sites (Fig 1A, B). Accordingly, without adaptation to high salt concentrations, a strong reduction of microbial processes by salinity would be expected. Increased salt tolerance of the communities induced by high salt concentrations could have partially offset the inhibition of microbial processes.

Based on the dose-response relationships observed in non-saline soils, without accounting for adaption to salinity the growth rate at a soil EC of 4 dS m^{-1} would be predicted to be ca. 10% of the growth in non-saline soils. In reality, along both gradients the growth rate measured in the most saline samples was about 20% of the growth rate measured in samples from the least saline end of the gradient (Fig. 3B). In the NV gradient, growth, as well as respiration, remained at a constant level until a soil EC of about 3 dS m^{-1}, after which it started to decline. In comparison, in the salt tolerance assays communities from non-saline soil experienced growth inhibition at suspension salinities equivalent to a soil EC of ca. 0.1 dSm^{-1} (Fig.1), which is below the generally held for threshold for saline soils (Richards, 1954). This suggests increasing community salt tolerance with higher salinity seems to be able to compensate for much of the loss in performance in moderately saline soils. It could also alleviate some of the loss in performance in the most saline soils, where bacterial growth is above the expectation based on inhibition of growth in non-saline soils. The increased community tolerance to salt provides insurance for continued functioning and allows for biogeochemical processes to be carried out at substantial rates in saline soils.

Conclusions

Along two environmental gradients of salinity, we could effectively quantify community-level trait distributions of salt tolerance. The local environmental conditions acted as a filter on communities, resulting in trait distributions that were matched to the environment and were indicative of a direct selective effect of salinity. We suggest using trait distributions along environmental gradients as an approach to distinguish direct effects of environmental factors on the communities

from spurious relationships, which could form the basis of a more predictive approach in microbial ecology.

We could also document large bacterial community differences along the salinity gradient. At higher salt concentrations bacteria became constrained by salinity, and the community compositions grew similar between the two gradients. In addition, there was large degree of overlap between the two gradients in terms of taxa whose abundance was positively correlated with increased salt tolerance, indicative of consistent changes in community composition with increased salt tolerance. These taxa could be used as biomarker sequences to infer the distribution of salt tolerance in the communities. Identifying useful biomarker sequences could make sequence information predictive of functional attributes of the community. By connecting filtered trait distributions to the composition of bacterial communities we were able to quantify the effect sizes that shifts in trait distributions have on community composition. Bacterial process rates in saline soils were increased relative to non-salt adapted communities at similar salinities, suggesting that a shift in community tolerance alleviates inhibiting effect of salinity on microbial performance.

References

Antwis, R.E., Griffiths, S.M., Harrison, X.A., Aranega-Bou, P., Arce, A., Bettridge, A.S., Brailsford, F.L., de Menezes, A., Devaynes, A., Forbes, K.M., Fry, E.L., Goodhead, I., Haskell, E., Heys, C., James, C., Johnston, S.R., Lewis, G.R., Lewis, Z., Macey, M.C., McCarthy, A., McDonald, J.E., Mejia-Florez, N.L., O'Brien, D., Orland, C., Pautasso, M., Reid, W.D.K., Robinson, H.A., Wilson, K., Sutherland, W.J., 2017. Fifty important research questions in microbial ecology. Fems Microbiology Ecology 93.

Bååth, E., Kritzberg, E., 2015. pH tolerance in freshwater bacterioplankton: Trait variation of the community as measured by leucine incorporation. Applied and Environmental Microbiology 81, 7411-7419.

Bárcenas-Moreno, G., Bååth, E., Rousk, J., 2016. Functional implications of the pH-trait distribution of the microbial community in a re-inoculation experiment across a pH gradient. Soil Biology & Biochemistry 93, 69-78.

Canfora, L., Bacci, G., Pinzari, F., Lo Papa, G., Dazzi, C., Benedetti, A., 2014. Salinity and bacterial diversity: To what extent does the concentration of salt affect the bacterial community in a saline soil? Plos One 9.

Fernández-Calviño, D., Arias-Estévez, M., Díaz-Raviña, M., Bååth, E., 2012. Assessing the effects of Cu and pH on microorganisms in highly acidic vineyard soils. European Journal of Soil Science 63, 571-578.

Fierer, N., Barberan, A., Laughlin, D.C., 2014. Seeing the forest for the genes: using rnetagenomics to infer the aggregated traits of microbial communities. Frontiers in Microbiology 5.

Geyer, K.M., Altrichter, A.E., Takacs-Vesbach, C.D., Van Horn, D.J., Gooseff, M.N., Barrett, J.E., 2014. Bacterial community composition of divergent soil habitats in a polar desert. Fems Microbiology Ecology 89, 490-494.

Green, J.L., Bohannan, B.J.M., Whitaker, R.J., 2008. Microbial biogeography: From taxonomy to traits. Science 320, 1039-1043.

Griffiths, R.I., Thomson, B.C., James, P., Bell, T., Bailey, M., Whiteley, A.S., 2011. The bacterial biogeography of British soils. Environmental Microbiology 13, 1642-1654.

Herlemann, D.P.R., Labrenz, M., Jurgens, K., Bertilsson, S., Waniek, J.J., Andersson, A.F., 2011. Transitions in bacterial communities along the 2000 km salinity gradient of the Baltic Sea. Isme Journal 5, 1571-1579.

Hollister, E.B., Engledow, A.S., Hammett, A.J.M., Provin, T.L., Wilkinson, H.H., Gentry, T.J., 2010. Shifts in microbial community structure along an ecological gradient of hypersaline soils and sediments. Isme Journal 4, 829-838.

Kamble, P.N., Gaikwad, V.B., Kuchekar, S.R., Bååth, E., 2014. Microbial growth, biomass, community structure and nutrient limitation in high pH and salinity soils from Pravaranagar (India). European Journal of Soil Biology 65, 87-95.

Lauber, C.L., Hamady, M., Knight, R., Fierer, N., 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Applied and Environmental Microbiology 75, 5111-5120.

Lauber, C.L., Strickland, M.S., Bradford, M.A., Fierer, N., 2008. The influence of soil properties on the structure of bacterial and fungal communities across land-use types. Soil Biology & Biochemistry 40, 2407-2415.

Logares, R., Lindstrom, E.S., Langenheder, S., Logue, J.B., Paterson, H., Laybourn-Parry, J., Rengefors, K., Tranvik, L., Bertilsson, S., 2013. Biogeography of bacterial communities exposed to progressive long-term environmental change. Isme Journal 7, 937-948.

Lozupone, C.A., Knight, R., 2007. Global patterns in bacterial diversity. Proceedings of the National Academy of Sciences of the United States of America 104, 11436-11440.

Morrissey, E.M., Franklin, R.B., 2015. Evolutionary history influences the salinity preference of bacterial taxa in wetland soils. Frontiers in Microbiology 6.

Oliverio, A.M., Bradford, M.A., Fierer, N., 2017. Identifying the microbial taxa that consistently respond to soil warming across time and space. Global Change Biology 23, 2117-2129.

Oren, A., 1999. Bioenergetic aspects of halophilism. Microbiology and Molecular Biology Reviews 63, 334-+.

Pandit, A.S., Joshi, M.N., Bhargava, P., Shaikh, I., Ayachit, G.N., Raj, S.R., Saxena, A.K., Bagatharia, S.B., 2015. A snapshot of microbial communities from the Kutch: one of the largest salt deserts in the World. Extremophiles 19, 973-987.

Rath, K.M., Maheshwari, A., Bengtson, P., Rousk, J., 2016. Comparative toxicity of salts to microbial processes in soil. Applied and Environmental Microbiology, AEM. 04052-04015.

Rath, K.M., Rousk, J., 2015. Salt effects on the soil microbial decomposer community and their role in organic carbon cycling: A review. Soil Biology & Biochemistry 81, 108-123.

Rengasamy, P., 2010. Soil processes affecting crop production in salt-affected soils. Functional Plant Biology 37, 613-620.

Richards, L.A., 1954. Diagnosis and improvement of saline and alkali soils. Soil Science 78, 154.

Rousk, J., Bååth, E., Brookes, P.C., Lauber, C.L., Lozupone, C., Caporaso, J.G., Knight, R., Fierer, N., 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil. Isme Journal 4, 1340-1351.

Rousk, J., Elyaagubi, F.K., Jones, D.L., Godbold, D.L., 2011. Bacterial salt tolerance is unrelated to soil salinity across an arid agroecosystem salinity gradient. Soil Biology & Biochemistry 43, 1881-1887.

Setia, R., Gottschalk, P., Smith, P., Marschner, P., Baldock, J., Setia, D., Smith, J., 2013. Soil salinity decreases global soil organic carbon stocks. Science of The Total Environment 465, 267-272.

Szabolcs, I., 1989. Salt-Affected Soils. C R C Press LLC.

Van Horn, D.J., Okie, J.G., Buelow, H.N., Gooseff, M.N., Barrett, J.E., Takacs-Vesbach, C.D., 2014. Soil microbial responses to increased moisture and organic resources

along a salinity gradient in a polar desert. Applied and Environmental Microbiology 80, 3034-3043.

Vavourakis, C.D., Ghai, R., Rodriguez-Valera, F., Sorokin, D.Y., Tringe, S.G., Hugenholtz, P., Muyzer, G., 2016. Metagenomic insights into the uncultured diversity and physiology of microbes in four hypersaline soda lake brines. Frontiers in Microbiology 7.

Ventosa, A., Fernandez, A.B., Leon, M.J., Sanchez-Porro, C., Rodriguez-Valera, F., 2014. The Santa Pola saltern as a model for studying the microbiota of hypersaline environments. Extremophiles 18, 811-824.

Wallenstein, M.D., Hall, E.K., 2012. A trait-based framework for predicting when and where microbial adaptation to climate change will affect ecosystem functioning. Biogeochemistry 109, 35-47.

Webb, C.T., Hoeting, J.A., Ames, G.M., Pyne, M.I., Poff, N.L., 2010. A structured and dynamic framework to advance traits-based theory and prediction in ecology. Ecology Letters 13, 267-283.

Wong, V.N.L., Dalal, R.C., Greene, R.S.B., 2008. Salinity and sodicity effects on respiration and microbial biomass of soil. Biology and Fertility of Soils 44, 943-953.

Xie, K.H., Deng, Y., Zhang, S.C., Zhang, W.H., Liu, J.R., Xie, Y.L., Zhang, X.Z., Huang, H., 2017. Prokaryotic Community Distribution along an Ecological Gradient of Salinity in Surface and Subsurface Saline Soils. Scientific Reports 7.

Zhong, Z.P., Liu, Y., Miao, L.L., Wang, F., Chu, L.M., Wang, J.L., Liu, Z.P., 2016. Prokaryotic community structure driven by salinity and ionic concentrations in plateau lakes of the Tibetan Plateau. Applied and Environmental Microbiology 82, 1846-1858.

Supplemental information

Fig. S1: Satellite image of the sampling sites along the two salinity gradients. The AG gradient was sampled in three transects at the northern shore of the lake (sampling points 120-127, 130-137 and 140-147). The NV gradient was sample in three transects at the southern shore of the lake (sampling points 150-157, 160-167 and 170-177). Source: Google Earth 7.1.8.3036 (image date 11/26/2015).
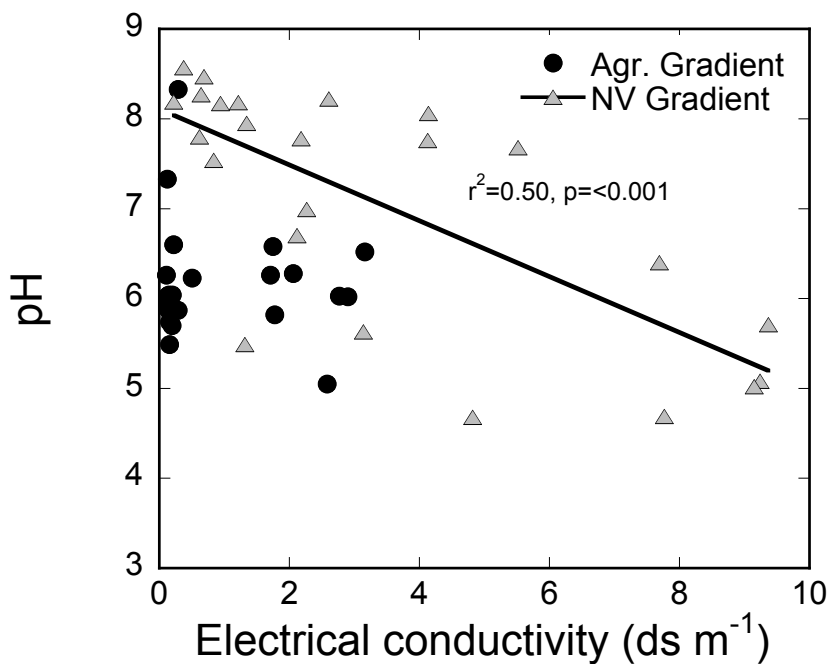
Fig.S2: Relationship between electrical conductivity (soil EC) and pH (soil pH) in a 1:5 soil:water mixture along the two salinity gradients. In the AG Gradient soil EC and soil pH were not correlated, whereas along the NV Gradient soil EC and soil pH were found to be significantly negatively correlated (r^2= 0.50, p<0.001).
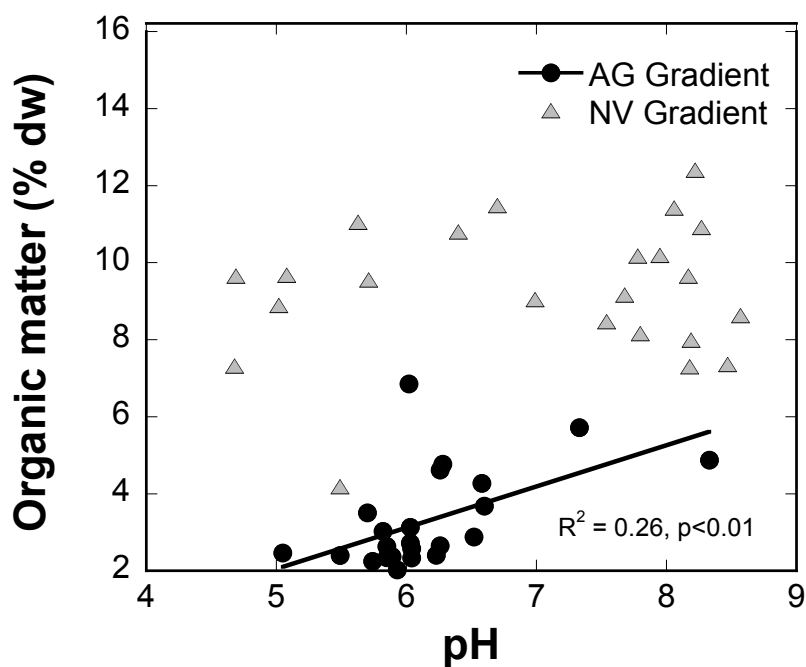
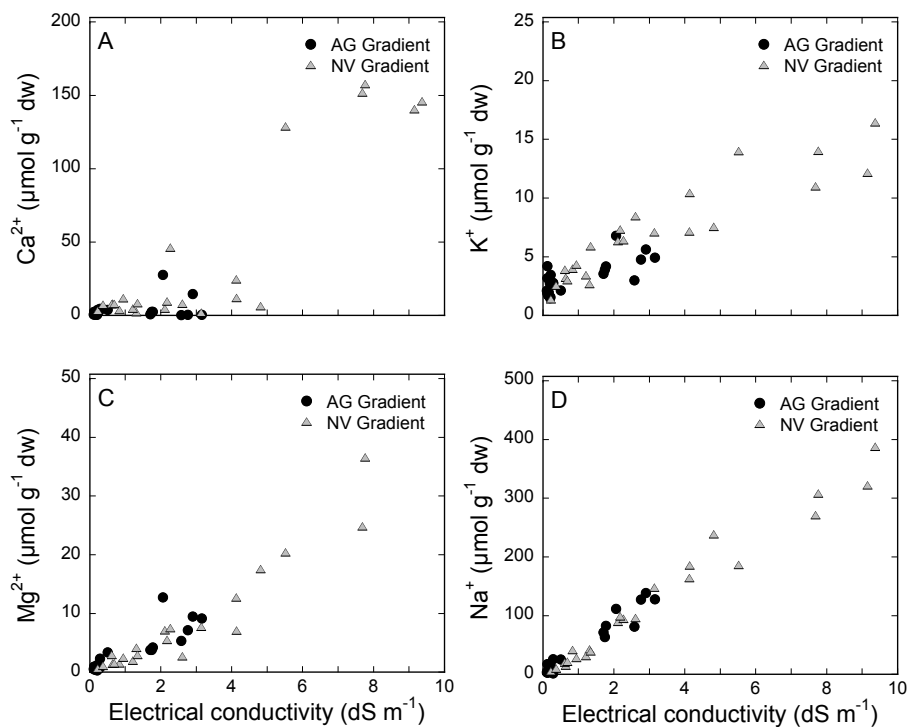Fig. S3: Relationship between pH and organic matter content (as % dry weight (dw) of soil) along the AG and the NV gradient.

Fig. S4: Concentrations of water-soluble cations (Ca^{2+}, K^+, Mg^{2+} and Na^+) along the salinity gradients
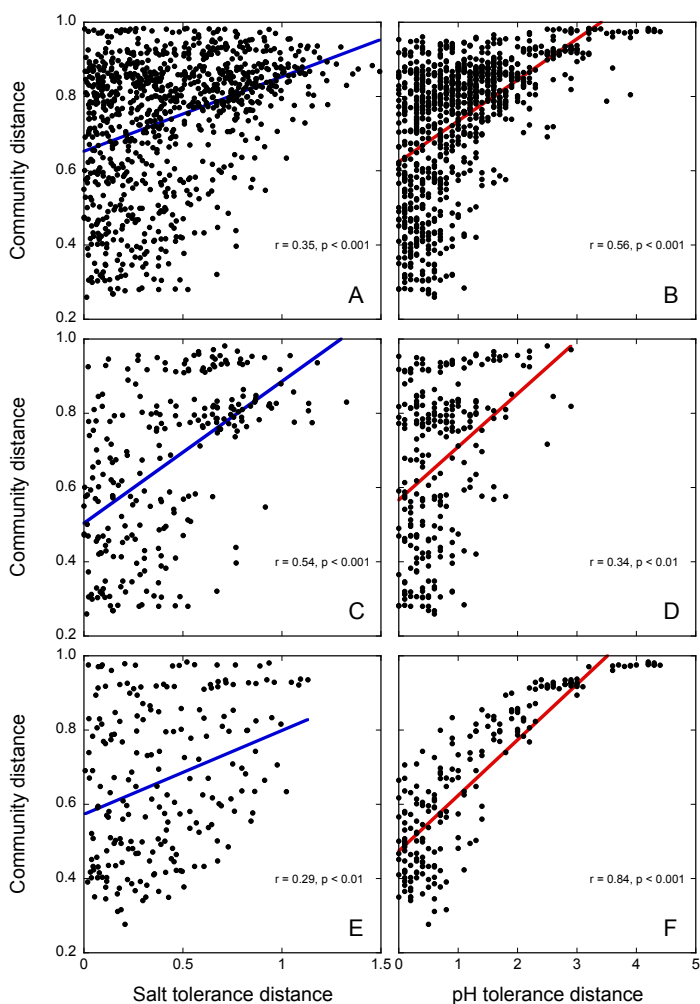
Fig. S5: Relationships between pairwise differences in community composition (Bray-Curtis dissimilarities) and community trait distributions (Euclidean distances). The fitted lines show linear regression curves between pairwise community distances and tolerance trait distances. Statistics (r) were calculated through Mantel tests. Panels A and B show the correlation between community distances and salt (A) and pH (B) tolerance distances for all samples in the dataset. Panels C and D include samples from the AG gradient only, panels E and F from the NV gradient.
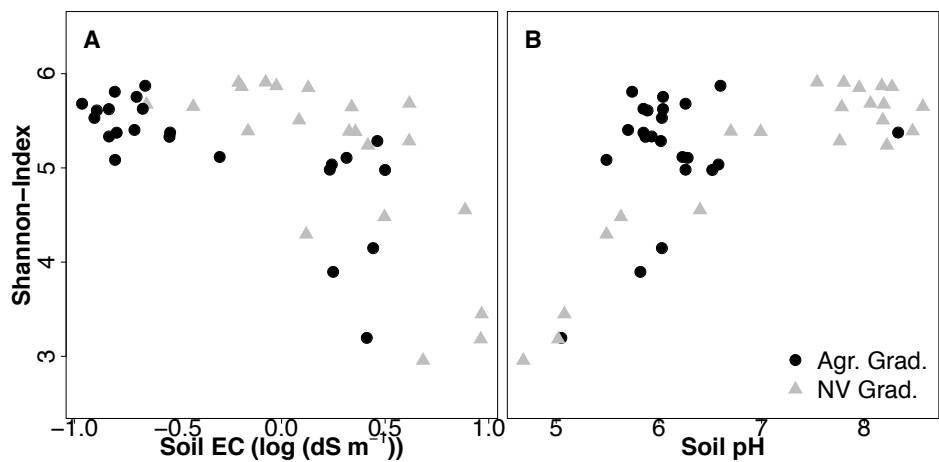
Fig. S6: Shannon diversity against (A) soil electrical conductivity (EC) and (B) soil pH.

Supplementary methods

Vegetation along gradients

On the northern shore gradient (henceforth the agricultural [AG] gradient), the first sampling point of each transect was located at the edge of the salt lake. The vegetation at sampling point 1 consisted of small halophilic shrubs with little ground cover. At sampling points 2-4 halophilic species were gradually replaced by grasses and small trees (*Allocasuarina sp.* and *Eucalytpus sp.*), while ground cover increased with distance from the lakeshore. Sampling points 5-8 were located in agricultural land used for wheat production. The distance covered by each transect of the AG gradient was ca. 200 m. The distance between transect A and B was ca. 130 m and between B and C ca. 560 m.

On the southern shore gradient (henceforth the natural vegetation [NV] gradient), sampling point 1 was also located at the lakeshore, with a vegetation consisting of mostly halophilic shrubs. At sampling point 2 vegetation consisted of grasses and *Allocasuarina sp.* From samplings points 3-6 *Eucalyptus sp.* became more common, with increasing ground cover. Sampling points 7-8 were covered with non-saline *Eucalyptus* woodland. Each transect of the NV gradient covered a distance of ca. 300 m from the lakeshore to the last sampling point. The distance between transects was ca. 300 m.

Characterization of gradients

After collection soil samples were sieved (<2.8 mm) and adjusted to 40% water holding capacity. Subsequently, electrical conductivity (soil EC) and pH (soil pH) were measured in a 1:5 soil: water mixture. Organic matter (OM) contents were obtained as loss-on-ignition (600°C, 12h).

Bacterial growth

Bacterial growth was measured as the incorporation of ^{3}H-labelled leucine into bacterial protein (Bååth, 1994; Bååth et al., 2001). Briefly, 2 µl of radioactively labelled leucine, ([3H]Leu, 185 MBq ml^{-1}, 2 TBq mmol^{-1}, Perkin Elmer) were added together with non-labelled leucine to the samples, resulting in a total concentration of 280 nM leucine. After 1 h incubation at room temperature growth was terminated by the addition of trichloroacetic acid. After a series of washing steps the amount of incorporated ^{3}H-label was determined through liquid scintillation.

Respiration and substrate-induced respiration (SIR)

Soil (1 g) was weighed into a 20 ml glass vial. The head-space was purged with pressurized air, sealed and incubated at 22 °C in the dark for ca. 12 h. Afterwards the CO_2 concentration in the headspace was analysed using a gas chromatograph (GC), equipped with a methanizer and a flame ionization detector. Background levels of CO_2 in pressurized air were subtracted from CO_2 levels measured in the sample headspace. Microbial biomass was determined through substrate-induced respiration (SIR), by adding 6 mg of glucose per g soil. After 2 h incubation, CO_2 was measured and converted to microbial biomass-C by using the relationship that 1 mg CO_2 h^{-1} produced corresponds to 20 mg biomass-C (Rousk et al., 2009).

DNA extraction, amplification and sequencing

Subsamples of each soil sample were freeze-dried and ground. DNA was extracted from portions of 250 mg of homogenized ground soil using the MoBio PowerSoil DNA Isolation Kit (Carlsbad, CA, USA) according to the manufacturer's recommendations. Extracted DNA was amplified using the 16S rRNA gene primer pair 515-F (5′-GTGCCAGCMGCCGCGGTAA-3′) and 806-R (5′-GGACTACHVGGGTWTCTAAT-3′), which included Illumina adapters and unique barcode sequences for each sample. PCR was performed with GoTaq® Hot Start PCR Master Mix (Promega, Madison, WI USA) in a 25 μL reaction. Thermal cycling consisted of an initial denaturation step at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C (45s), annealing at 50°C (30 s), extension at 70°C (90 s), and a final extension at 72°C for 10 min. The amplified DNA was sequenced using a Illumina MiSeq platform (Illumina, San Diego, CA USA).

Sequences were processed using the UPARSE pipeline (Edgar, 2013) as described in Ramirez et al. (2014). Sequences were quality filtered and clustered de novo into operational taxonomic units (OTUs) at a 97% similarity level. Taxonomic information was assigned to OTUs using the 16S rRNA Greengenes database (McDonald et al., 2012). To correct for differences in sequencing depth samples were rarefied to 10 000 reads. Samples with < 10 000 reads and OTUs that were observed fewer than 10 times across all samples were excluded from downstream analyses. This removed 4 out of 48 samples and 8928 of 12326 OTUs.

Data analysis

In samples in which bacterial growth was inhibited only by increasing salinity, dose-response relationships were established using a logistic model, $Y= c/[1+ e^{b(x-a)}]$, where Y is the leucine incorporation rate, x is the logarithm of the suspension EC, a is the $logIC_{50}$, c is the bacterial growth rate in the control without added salt, and b is a slope parameter indicating the rate of inhibition. In samples in which growth was inhibited by both increasing and decreasing salinity from its growth

optimum a double logistic model (Bååth and Kritzberg, 2015) was used: $Y = c_{opt}$ / $(1 + exp[b_{low\text{-}EC} (x - a_{(low\text{-}EC)})]) + c_{opt}$ / $(1 + exp[b_{high\text{-}EC}(x - a_{high\text{-}EC})]) - c_{opt}$, with c_{opt} the growth rate at optimal suspension EC, b the slope indicating the rate of decrease towards higher or lower suspension EC, and a the $logIC_{50}$ towards higher and lower suspension EC. To estimate the pH tolerance of the communities, the same double-logistic model was used, with suspension pH replacing suspension EC as the predictor variable. The suspension pH at which bacterial growth reached its optimum (pH_{opt}) in each sample was used as an indicator of the community-level trait distribution of pH tolerance. Kaleidagraph 4.5.0 for Mac (Synergy software) was used to fit the logistic and double logistic models. Linear regression models were used to test for significant correlations between community-level trait indicators ($logIC_{50}$ and pH_{opt}) and soil EC and pH.

The diversity of each sample was determined by calculating the Shannon diversity index. Multivariate statistics were performed in the R environment version 3.3.1 (R Core Team, 2016) using the 'vegan' package (Oksanen et al., 2016). The differences in overall community composition between samples were calculated using the Bray-Curtis dissimilarity index after Hellinger transformation (Legendre and Gallagher, 2001). A constrained ordination was performed by db-RDA (distance-based redundancy analysis) using *capscale* (vegan) with $logIC_{50}$, pH_{opt} and gradient as constraining variables. The significance of constraining variables was tested with a permutation test (number of permutations =10 000) using the *anova* function of the vegan package. Correlations between community composition and distributions of salt and pH tolerance were tested using Mantel tests between Bray-Curtis distance matrices of community composition and Euclidean distance matrices of trait distributions. To identify important OTUs correlated with trait distributions we selected OTUs with a relative abundance of $\geq$ 1% in at least one sample. For these OTUs we calculated Spearman's rank correlations with both $logIC_{50}$ and pH_{opt}. OTUs with a Spearman's rank correlation coefficient (ρ) of ≥ 0.5 or ≤ -0.5 were selected as being positively or negatively correlated with a certain trait.

To test for significant correlations between diversity and environmental variables, multiple linear regressions ($\alpha = 0.05$) were performed for each gradient followed by an ANOVA using type II Sums of Squares, with Shannon diversity as the dependent variable, and the logarithm of EC and pH as independent variables. Multiple linear regression models followed by an ANOVA using type II Sums of Squares were also used to test for significant relationships between environmental variables and functional parameters. The logarithm of the soil EC (logEC) and pH were used as predictor variables, and biomass, respiration and bacterial growth as independent variables. Multiple linear regression models were performed in the R environment version 3.3.1 (R Core Team, 2016).

Supplementary references

Bååth, E., 1994. Thymidine and leucine incorporation in soil bacteria with different cell size. Microbial Ecology 27, 267-278.

Bååth, E., Kritzberg, E., 2015. pH tolerance in freshwater bacterioplankton: Trait variation of the community as measured by leucine incorporation. Applied and Environmental Microbiology 81, 7411-7419.

Bååth, E., Pettersson, M., Söderberg, K.H., 2001. Adaptation of a rapid and economical microcentrifugation method to measure thymidine and leucine incorporation by soil bacteria. Soil Biology & Biochemistry 33, 1571-1574.

Edgar, R.C., 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nature Methods 10, 996-+.

Legendre, P., Gallagher, E.D., 2001. Ecologically meaningful transformations for ordination of species data. Oecologia 129, 271-280.

McDonald, D., Price, M.N., Goodrich, J., Nawrocki, E.P., DeSantis, T.Z., Probst, A., Andersen, G.L., Knight, R., Hugenholtz, P., 2012. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. Isme Journal 6, 610-618.

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E., Wagner, H., 2016. vegan: Community Ecology Package, R package version 2.4-1.

R Core Team, 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Ramirez, K.S., Leff, J.W., Barberan, A., Bates, S.T., Betley, J., Crowther, T.W., Kelly, E.F., Oldfield, E.E., Shaw, E.A., Steenbock, C., Bradford, M.A., Wall, D.H., Fierer, N., 2014. Biogeographic patterns in below-ground diversity in New York City's Central Park are similar to those observed globally. Proceedings of the Royal Society B-Biological Sciences 281.

Rousk, J., Brookes, P.C., Bååth, E., 2009. Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. Applied and Environmental Microbiology 75, 1589-1596.

Supplementary tables to Paper IV

Table S1: OTUs found to be positively correlated with community salt tolerance along the AG and NV gradient. Selected were OTUs with a maximal abundance of >1% and a Spearman's correlation coefficient ρ of >0.5.

AG Gradient			
OTU ID [1]	ρ [2]	Max. abund [3].	Taxonomy [4]
OTU_79	0.84	6.5	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; unclassified; unclassified; unclassified; unclassified
OTU_433	0.81	1.8	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Salinisphaerales; f__Salinisphaeraceae; g__Salinisphaera; s__
OTU_119	0.80	1.8	k__Bacteria; p__Gemmatimonadetes; c__Gemm-5; o__; f__; g__; s__
OTU_279	0.79	7.9	k__Bacteria; p__Bacteroidetes; c__Flavobacteriia; o__Flavobacteriales; f__Flavobacteriaceae; g__Gillisia; s__
OTU_137	0.78	1.3	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhizobiales; f__Aurantimonadaceae; g__; s__
OTU_336	0.78	2.8	k__Bacteria; p__Bacteroidetes; c__[Rhodothermi]; o__[Rhodothermales]; f__[Balneolaceae]; g__KSA1; s__
OTU_396	0.77	1.1	k__Bacteria; p__Bacteroidetes; c__[Rhodothermi]; o__[Rhodothermales]; f__Rhodothermaceae; g__Rubricoccus; s__
OTU_407	0.77	1.5	k__Bacteria; p__BRC1; c__PRR-11; o__; f__; g__; s__
OTU_944	0.76	1.5	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Xanthomonadales; f__Xanthomonadaceae; g__; s__
OTU_197	0.76	1.1	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Xanthomonadales; f__Xanthomonadaceae; unclassified; unclassified
OTU_736	0.76	1.1	k__Bacteria; p__Bacteroidetes; c__[Rhodothermi]; o__[Rhodothermales]; f__Rhodothermaceae; g__; s__

OTU_225	0.75	3.5	k__Bacteria; p__Bacteroidetes; c__Flavobacteriia; o__Flavobacteriales; f__Flavobacteriaceae; unclassified; unclassified
OTU_420	0.75	1.0	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Alteromonadales; f__Alteromonadaceae; g__; s__
OTU_755	0.75	3.2	k__Bacteria; p__Bacteroidetes; c__Flavobacteriia; o__Flavobacteriales; f__Flavobacteriaceae; g__Gramella; s__
OTU_13435	0.75	1.6	k__Bacteria; p__Proteobacteria; c__Deltaproteobacteria; o__GMD14H09; f__; g__; s__
OTU_1582	0.71	1.2	k__Bacteria; p__Bacteroidetes; c__Flavobacteriia; o__Flavobacteriales; f__Flavobacteriaceae; g__Gramella; s__
OTU_468	0.71	4.2	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Alteromonadales; f__Alteromonadaceae; g__Marinobacter; unclassified
OTU_671	0.71	10.5	k__Bacteria; p__Bacteroidetes; c__Cytophagia; o__Cytophagales; f__Flammeovirgaceae; g__; s__
OTU_397	0.70	7.9	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Xanthomonadales; f__Xanthomonadaceae; g__; s__
OTU_154	0.70	2.3	k__Bacteria; p__Bacteroidetes; c__Cytophagia; o__Cytophagales; f__Flammeovirgaceae; g__; s__
OTU_523	0.70	1.4	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Xanthomonadales; f__Xanthomonadaceae; g__; s__
OTU_124	0.69	3.6	k__Bacteria; p__Bacteroidetes; c__[Rhodothermi]; o__[Rhodothermales]; f__[Balneolaceae]; g__KSA1; s__
OTU_467	0.65	3.4	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; unclassified; unclassified; unclassified; unclassified
OTU_103	0.65	14.8	k__Bacteria; p__Bacteroidetes; c__[Rhodothermi]; o__[Rhodothermales]; f__[Balneolaceae]; g__KSA1; s__
OTU_429	0.61	2.8	k__Bacteria; p__Bacteroidetes; c__Flavobacteriia; o__Flavobacteriales; f__Cryomorphaceae; g__Owenweeksia; s__
OTU_532	0.61	1.4	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Alteromonadales; f__Alteromonadaceae; g__; s__
OTU_9051	0.60	1.5	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; unclassified; unclassified; unclassified; unclassified
OTU_676	0.60	1.9	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhizobiales; f__Hyphomicrobiaceae; g__Devosia; s__
OTU_339	0.60	2.6	k__Bacteria; p__Gemmatimonadetes; c__Gemm-3; o__; f__; g__; s__
OTU_111	0.60	1.5	k__Bacteria; p__Bacteroidetes; c__[Rhodothermi]; o__[Rhodothermales]; f__[Balneolaceae]; unclassified; unclassified
OTU_267	0.59	3.7	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Salinisphaerales; f__Salinisphaeraceae; g__; s__
OTU_17369	0.59	6.2	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Sphingomonadales; f__Sphingomonadaceae; g__; s__
OTU_1052	0.59	21.8	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Xanthomonadales; f__Sinobacteraceae;

			g__Alkanibacter; s__difficilis
OTU_327	0.58	1.0	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Sphingomonadales; f__Sphingomonadaceae; g__Kaistobacter; s__
OTU_5388	0.58	7.3	k__Bacteria; p__Bacteroidetes; c__Flavobacteriia; o__Flavobacteriales; f__Cryomorphaceae; g__; s__
OTU_441	0.57	2.7	k__Bacteria; p__Proteobacteria; c__Deltaproteobacteria; o__GMD14H09; f__; g__; s__
OTU_675	0.56	6.0	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Oceanospirillales; f__Alcanivoracaceae; g__Alcanivorax; unclassified
OTU_768	0.55	1.5	k__Bacteria; p__Bacteroidetes; c__[Saprospirae]; o__[Saprospirales]; f__Chitinophagaceae; g__; s__
OTU_332	0.55	2.1	k__Bacteria; p__Bacteroidetes; c__Flavobacteriia; o__Flavobacteriales; f__Flavobacteriaceae; g__Salinimicrobium; s__
OTU_800	0.55	1.6	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhizobiales; f__Hyphomicrobiaceae; g__; s__
OTU_50	0.55	1.1	k__Bacteria; p__Firmicutes; c__Bacilli; o__Bacillales; f__Sporolactobacillaceae; g__; s__
OTU_31	0.54	2.6	k__Bacteria; p__Chlorobi; c__Ignavibacteria; o__Ignavibacteriales; f__Ignavibacteriaceae; g__; s__
OTU_253	0.53	3.5	k__Bacteria; p__Bacteroidetes; c__[Rhodothermi]; o__[Rhodothermales]; f__[Balneolaceae]; g__Balneola; s__
OTU_167	0.51	1.4	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Xanthomonadales; f__Xanthomonadaceae; unclassified; unclassified
OTU_902	0.51	1.2	k__Bacteria; p__TM6; c__SJA-4; o__; f__; g__; s__
OTU_1290	0.51	1.4	k__Archaea; p__Euryarchaeota; c__Halobacteria; o__Halobacteriales; f__Halobacteriaceae; g__Halococcus; s__

NV Gradient

OTU ID	ρ	Max. abundance	Taxonomy
OTU_79	0.69	41.2	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; unclassified; unclassified; unclassified; unclassified
OTU_50	0.68	9.9	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Salinisphaerales; f__Salinisphaeraceae; g__Salinisphaera; s__

OTU_267	0.66	6.1	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; unclassified; unclassified; unclassified; unclassified
OTU_10249	0.65	3.3	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Xanthomonadales; f__Xanthomonadaceae; unclassified; unclassified
OTU_119	0.64	5.0	k__Bacteria; p__Bacteroidetes; c__[Rhodothermi]; o__[Rhodothermales]; f__[Balneolaceae]; g__KSA1; s__
OTU_288	0.61	1.8	k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Sphingomonadales; f__Erythrobacteraceae; g__; s__
OTU_639	0.58	2.9	k__Bacteria; p__Chloroflexi; c__Ktedonobacteria; o__B12-WMSP1; f__; g__; s__
OTU_5818	0.58	1.1	k__Bacteria; p__Actinobacteria; c__Actinobacteria; o__Actinomycetales; unclassified; unclassified; unclassified
OTU_8324	0.57	2.9	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Xanthomonadales; f__Xanthomonadaceae; g__Luteibacter; s__rhizovicinus
OTU_17369	0.57	3.7	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Xanthomonadales; f__Xanthomonadaceae; unclassified; unclassified
OTU_4189	0.57	5.0	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; unclassified; unclassified; unclassified; unclassified
OTU_668	0.55	2.2	k__Bacteria; p__Bacteroidetes; c__[Rhodothermi]; o__[Rhodothermales]; f__[Balneolaceae]; unclassified; unclassified
OTU_124	0.54	11.0	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Xanthomonadales; f__Xanthomonadaceae; g__; s__
OTU_433	0.53	1.7	k__Bacteria; p__Gemmatimonadetes; c__Gemm-5; o__; f__; g__; s__
OTU_407	0.53	2.2	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Xanthomonadales; f__Xanthomonadaceae; unclassified; unclassified
OTU_823	0.52	1.4	k__Bacteria; p__Planctomycetes; c__Planctomycetia; o__Gemmatales; f__Isosphaeraceae; g__; s__
OTU_319	0.52	5.5	k__Bacteria; p__Proteobacteria; c__Gammaproteobacteria; o__Alteromonadales; f__Alteromonadaceae; g__Marinobacter; unclassified
OTU_253	0.51	11.9	k__Bacteria; p__Bacteroidetes; c__[Rhodothermi]; o__[Rhodothermales]; f__[Balneolaceae]; unclassified; unclassified
OTU_31	0.51	1.1	k__Bacteria; p__Firmicutes; c__Bacilli; o__Bacillales; f__Sporolactobacillaceae; g__; s__

1 Number assigned to each operational taxonomic unit (OTU)

2 Spearman rank correlation coefficient ρ

3 Maximum abundance of each OTU in the gradient in % of reads

4 Taxonomy assigned to OTU: k= kingdom, p = phylum, c= class, o=order, f=family, g=genus, s=species

Bacterial and fungal growth and biomass along natural gradients of soil salinity

Rath, Kristin M.[1,2], Murphy, Daniel[3] and Rousk, Johannes[1]

[1] Section of Microbial Ecology, Department of Biology, Lund University, Lund, Sweden

[2] Centre for Environmental and Climate Research (CEC), Lund University, Lund, Sweden

[3] School of Agriculture and Environment, University of Western Australia, Perth, WA, Australia

Abstract

The land area that is affected by high soil salinity is increasing globally. Assessments of the status of the microbial community in saline soils have frequently been based on microbial biomass estimates, which might not accurately reflect microbial process rates in soil. Moreover, assessments of the relative importance of major decomposer groups of bacteria and fungi in saline soils to date have been inconclusive. In this study we use soil samples from two salinity gradients, which we supplied with plant material to compensate for differences in organic matter content between soils and isolate the effect of salinity. The research aims were to (i) compare the response to salinity of biomass, which was estimated based on both PLFA and qPCR measurements, to that of respiration and microbial growth, (ii) compare the response of bacteria and fungi and (iii) assess the response of microbial parameters to alleviation of low organic matter contents in saline soils. We found that generally biomass was less negatively affected by salinity than bacterial growth and respiration, and was not related to rates of processes in soil. While bacterial growth was strongly negatively affected by salinity, fungal growth was similar in soils of all salinities, indicating a low impact of salinity on fungal performance. Organic matter additions increased process rates in saline soils and alleviated some of the negative impact of salinity on respiration and growth. Overall, this study represents the first assessment of microbial growth along natural salinity gradients.

1. Introduction

Large areas of land are affected by high salt concentrations globally, and the extent of salt-affected areas has increased due to land-use change and agricultural management (Rengasamy, 2006). Commonly, a soil is defined as saline if the electrical conductivity measured in a saturated paste surpasses 4 dS m^{-1} (Richards, 1954). In Australasia, a region in which soil salinization is particularly common, about 360 million hectares of soils are affected by high salt concentrations (Szabolcs, 1989; Rengasamy, 2006). Salinity negatively affects organisms by restricting water availability as a result of low osmotic potentials in soils and through ion toxicity. Consequently, salinity exerts an inhibitory effect on the microbial community in soils in the short-term (Rath et al., 2016), as well as a long-term exposure to salinity (Yuan et al., 2007; Rath and Rousk, 2015).

The effect of salinity on the status of the microbial community can be assessed in different ways. One of the most commonly measured parameters is the microbial biomass, with the expectation that salinity would result in a decreased microbial biomass. However, soil organic matter (OM) contents also often decline with increasing salinity, which means that decreases in microbial biomass could also be a result of declining OM content rather than salinity itself. Biomass measurements per unit OM in saline soils often do not follow a clear relationship with salinity (Rath and Rousk, 2015). Moreover, biomass does not correlate well with microbial growth or mineralization rates (Rousk, 2016). Since biomass is a function of production of new biomass, mortality and turnover of necromass, biomass is not a quickly responding factor to environmental change, but could capture long-term effects of salinization.

Different methods have been established for the estimation of microbial abundance in soils. One frequently used method is based on the concentration of fatty acids derived from phospholipids (phospholipid-fatty acids, PLFA), a group of lipids found in cell membranes, which can also yield information about the microbial community composition in the soil (Frostegård and Bååth, 1996). Another way to estimate microbial abundances are methods that quantify the abundance of DNA through quantitative polymerase chain reaction (qPCR). Through the use of group-specific primers, the abundance of selected taxonomic groups can be resolved. DNA and lipids are thought to differ greatly in their turnover time, with lipids having a much longer residence time in soil than nucleic acids (Malik et al., 2015), which could result in different degrees of sensitivity of lipid and DNA-based biomass measurements.

In contrast to biomass stocks, the microbial growth rate should yield a better estimate of the contribution of the microbial community to ecosystem functioning

(Rousk, 2016). The build-up of new biomass scales with many other processes carried out by soil microorganisms. However, the relationship between microbial growth and mineralization rates is determined by the microbial growth efficiency, i.e. the ratio between substrate used for the production of new biomass and substrate that is respired. The relative impact of salinity on microbial anabolism, i.e. growth, and catabolic processes, such as respiration, determines the fate of substrate in the system.

Bacteria and fungi are the two main groups of decomposers of organic matter (OM) in the soil. Hypersaline habitats are thought to be dominated by prokaryotic organisms (Horikoshi and Grant, 1998), which is mainly based on data collected from hypersaline aquatic systems, where communities are better studied than communities in highly saline soils. However, fungi also have the capability to withstand extremely low water potentials caused by desiccation or high concentrations of solutes (Grant, 2004). It is unclear whether bacteria or fungi in soil would be more sensitive towards increased soil salinization, and both higher fungal salt sensitivity (Sardinha et al., 2003; Chowdhury et al., 2011) and bacterial salt sensitivity (Wichern et al., 2006; Kamble et al., 2014; Rath et al., 2016) have been reported. Shifts in the fungal/bacterial ratio in response to salinity could have implications for C cycling in soil, as fungi are thought to be able to decompose more complex organic material, while at the same time fungal necromass is thought to have a longer turnover time in soil than bacterial residues (Six et al., 2006; Strickland and Rousk, 2010).

The aim of this study was to use two natural gradients of salinity to determine the impact of salinity on the microbial community. Specifically, our objectives were to (1) compare the effect of soil salinity on the bacterial and the fungal part of the microbial community, (2) assess the response of microbial biomass using measures based on two components of living cells with different residence times in soils and (3) determine the response of growth and mineralization rates to salinity. The soils we used came from two salinity gradients located at the shore of a salt lake in Western Australia. Along these gradients of salinity, plant growth was strongly reduced in saline soils, which would have resulted in lower OM input into saline soils. To compensate for differences in OM quantity and quality of soils of different salinities and isolate the effect soil salinity along salinity gradients from that of soil OM content, we supplied soils with additional plant material and incubated them for a period of weeks. We hypothesized that (1) salinity would have a greater impact on growth and respiration rates than on biomass measurements, (2) that OM addition in the form of plant material would reduce the inhibition of salinity over the course of the experiment, and (3) that fungi would have greater resistance to salinity than bacteria based on earlier results obtained from short-term inhibition assays (Rath et al., 2016).

2. Material and methods

2.1. Sample collection and preparation

Samples were collected from two salt gradients located along Lake O'Connor in Western Australia. One gradient was located on the northern shore of the lake (32° 28'S, 119°12'E), the other gradient on the southern shore of the lake (32°29'S, 119°13'E) (Fig. S1). The distance between the two gradients was ca. 2 km. Each gradient was sampled in 3 transects of 8 sampling points, resulting in a total of 24 samples collected for each gradient. Each of the transects began at the shore of the salt lake. In the northern shore gradient (henceforth "AG" (agricultural) gradient), sampling points 5-8 were located in agricultural land used for wheat production. The AG transects each covered a distance of roughly 200 m. The distance between transects A and B was 130 m and between B and C 560 m. On the southern shore gradient (henceforth "NV" (natural vegetation) gradient), samplings points 3-8 were located in eucalyptus woodland, with increasing ground cover with distance from the salt lake. Each transect of the NV gradient covered a distance of roughly 300 m from the lakeshore to the last sampling point. The distances between transects were ca. 300 m.

After collection, soil samples were sieved (<2.8 mm) and adjusted to 40% water holding capacity. Subsequently, electrical conductivity and pH were measured in a 1:5 soil:water mixture. Organic matter (OM) contents were obtained as loss-on-ignition of dried soils (600°C, 12h).

2.2. Biological parameters

2.2.1. Biomass (PLFA)

Portions of soils were freeze-dried, followed by extraction of fatty acids according to Frostegård and Bååth (1996) in a mixture of chloroform, methanol and citrate buffer (pH = 4). On pre-packed silica columns fatty acids were fractionated into neutral, glycol- and polar lipids (including phospholipids) through subsequent eluations with chloroform, acetone and methanol. The polar lipid fraction was then dried under a stream of N_2 gas and methyl nonadecanoate fatty acid (19:0) was added as an internal standard. Phospholipids were methylated and separated on a gas chromatograph equipped with a flame ionization detector. The sum of the following PLFAs was used a measure of the bacterial biomass: i14:0, i15:0, a15:0, 15:0, i16:0, 10Me16:0, i17:0, a17:0, cy17:0, 17:0, br18:0, 10Me17:0, 18:1ω7, 10Me18:0 and cy19:0, while 18:2ω6,9 was used as a measure of fungal biomass (Frostegård and Bååth, 1996).

2.2.2. Bacterial growth

The bacterial growth rate was estimated by measuring the incorporation of ^{3}H-labelled leucine (Leu) into bacteria extracted from soil according to Bååth (1994) and Bååth et al. (2001). Soil (1 g) was mixed with 20 ml of water followed by a 10 min centrifugation step at 1000 g. From the resulting bacterial suspension a 1.5 ml subsample was used to measure bacterial growth. Since previous experiments had shown that growth in the most saline soils was inhibited when salinities where lowered by the addition of distilled water to create the soil suspension, bacterial growth in those soils was measured at a range of salinities through the addition of different concentrations of NaCl solutions to the soil suspension. The growth rate at the optimum salinity was then used to represent the growth rate in those soils. Tritiated leucine (2 µl [3]H Leu, 37 MBq ml^{-1}, 5.74 TBq mmol^{-1}; Perkin Elmer, UK) was added to the suspension together with non-labeled Leu, resulting in a final concentration of 275 nM Leu. After a 1 h incubation at 18 °C in the dark bacterial growth was terminated by the addition of 100% trichloroacetic acid. After a series of washing steps (Bååth et al., 2001) the amount of incorporated radioactive label was measured using liquid scintillation.

2.2.3. Fungal growth

The fungal growth rate was measured as the incorporation of ^{14}C-labelled acetate into ergosterol (Bååth, 2001). Briefly, 1.95 ml of water were added to 1 g of soil and mixed to create a soil slurry. To the slurry 20 µl 1-[^{14}C] acetic acid (sodium salt, 37 MBq ml^{-1}, 2.10 GBq mmol^{-1}, Perkin Elmer), and 30 µl of unlabeled 16 mM acetate were added, resulting in a final concentration of 220 µM acetate. Following a 5 h incubation at 18°C, fungal growth was terminated by adding 0.5 ml 10% formalin. Ergosterol was extracted from soil using 10% KOH in methanol. High-performance liquid chromatography (HPLC) combined with a UV detector (280 nm) was used to separate and quantify ergosterol. The radioactivity incorporated into ergosterol was measured using liquid scintillation.

2.2.4. Respiration

Soil (1 g) was weighed into a 20 ml glass vial. The head-space was purged with pressurized air, sealed and incubated at 18 °C in the dark for ca. 4 h. Afterwards the CO_2 concentration in the headspace was analysed using a gas chromatograph (GC), equipped with a methanizer and a flame ionization detector. Background levels of CO_2 in pressurized air were subtracted.

2.2.5 Quantitative polymerase-chain-reaction (qPCR)

DNA was extracted from 200 µg freeze-dried and ground soil using the PowerSoil® DNA Isolation Kit (MoBio). To quantify the abundance of bacterial and fungal gene sequences in soil, qPCR was used. Regions of the bacterial 16S rRNA gene were targeted with the universal bacterial primer pair Eub338 (5'-ACTCCTACGGGAGGCAGCAG-3') and Eub518 (5'-ATTACCGCGGCTGCTGG-3') (Fierer et al., 2005). The fungal ITS region was targeted using ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and 5.8S (5'-CGCTGCGTTCTTCATCG-3') (Fierer et al., 2005). Unknown samples were run against 4-fold dilution curves of standards consisting of genomic DNA extracted from cultures of *Bacillus subtilis* or *Serpula lacrymans*, respectively. DNA concentrations of the standards were measured fluorometrically using the Quant-iTTM PicoGreen® dsDNA kit (Invitrogen).

Samples were run in triplicates. Each reaction mixture had a total volume of 20 µl and contained around 1 ng of template DNA, 0.3 µM of each bacterial primer or 0.5 µM of each fungal primer, and 10 µl of 2X Fast Start Universal SYBR Green Master (Rox) (Roche). The qPCR was performed on a Stratagene Mx3000P (La Jolla, CA, USA). The program for bacterial qPCR consisted of an initial 10 min incubation at 95°C, followed by 40 cycles of denaturation for 15 s at 95°C and annealing and elongation for 60 s at 60°C. Amplification conditions for fungal qPCR were 10 min at 95°C, followed by 40 cycles of denaturation for 60 s at 95°C, annealing for 30 s at 53°C and elongation for 60 s at 72°C. The amplification program was followed by a dissociation curve. Amplification curves were analyzed using MxPro-Mx3005P v.4.10 (Stratagene, 2007).

2.3. Incubation experiment

To make up for possible differences in organic matter contents between sites of different salinity, samples from two transects of each gradient were incubated in microcosms with plant material in the form of 15 mg g^{-1} 1:1 alfalfa-wheat straw mixture. For each sample, duplicate microcosms were prepared. The number of samples was reduced from the full set to reduce sampling load during the incubation experiment. The selected samples covered the whole range of salinities observed along the gradients. The microcosms were kept at a constant temperature of 18°C and respiration, bacterial growth and fungal growth were measured before the start of the incubation with plant material, as well as on day 1, 2, 4, 7 and 17 (t0, 1, 2, 3 and 4) after substrate addition. qPCR was carried out on samples before the start of the experiment and at day 17 after substrate addition.

Differences in microbial biomass between the time points on day 1 and day 17 during the incubation experiment were tested using a paired t-test with unequal variances. The effects of the environmental variables of salinity and pH on biomass and cumulative respiration and growth were tested using a multiple linear regression model.

The PLFA profile of the samples from the time points on day 1 and day 17 was visualized using Redundancy Analysis (RDA) with salinity, pH, organic matter content and a dummy variable for timepoint as constraining variables. All four of the included variables explained a significant proportion of the variation in the dataset.

Results

3.1. Soil characteristics

The electrical conductivity (EC) along the salinity gradients ranged from ca. 0.1 to ca. 3 dS m-1 measured in a 1:5 soil water mixture along the AG gradient, and from ca. 0.3 to 10 dS m-1 along the NV gradient. Soil pH ranged from ca. 5.1 to 8.3 along the AG gradient with the majority of sites having a pH around 6, and from ca. 4.7 to 8.6 along the NV gradient. Soil OM content along the AG gradient ranged from 2% to 7% dry weight, and from 4% to 15% along the NV gradient. There was no correlation between OM content and soil EC along either gradient. Water holding capacity ranged from 17% to 72% dry weight along the AG gradient and from 31% to 78% dry weight along the NV gradient.

3.2. PLFA biomass

In the incubation experiment, total PLFA biomass (t = 2.5, $p < 0.05$) and bacterial PLFA biomass (t = 2.8, $p < 0.01$) were significantly higher on day 17 than on day 1 (Fig. 1A, B). Fungal PLFA biomass however did not change significantly between day 17 and day 1 (t = 1.0, p = 0.3) (Fig. 1C). The ratio of fungal/bacterial PLFA also did not change during incubation with plant material (t= 0.7, p = 0.5) (Fig. 1D). Along the AG gradient on day 1 total and bacterial biomass were significantly negatively correlated with salinity ($R^2 = 0.53$, $p < 0.01$ and $R^2 = 0.60$, $p < 0.001$), whereas at day 17 only total biomass was significantly correlated with salinity ($R^2 = 0.26$, $p < 0.05$) (Fig. 1A,B). Fungal biomass was not correlated with salinity (Fig. 1C). Along the NV gradient, no significant correlation between salinity and the PLFA biomass for any of the different decomposer groups was found. The ratio of fungal/bacterial PLFAs was not correlated with salinity along either gradient (Fig. 1D).
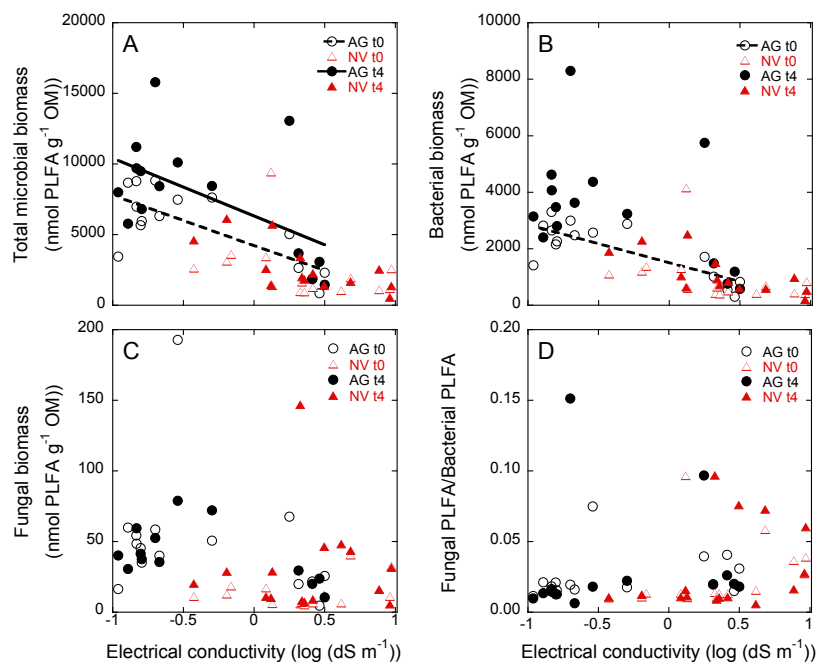
Fig. 1
Total biomass (A), bacterial biomass (B), fungal biomass (C) and the ratio of fungal to bacterial biomass (D) along the AG and NV salinity gradients on day 1 (t0) and on day 17 (t4) after addition of plant material to soil. Outlier values with fungal biomass > 300 nmol PLFA g^{-1} OM have been excluded from panels C and D.

3.3. PLFA composition

The PLFA profile of the samples from the time points on day 1 and day 17 was visualized using Redundancy Analysis (RDA) with salinity, pH, organic matter content and a dummy variable for time point as constraining variables (Fig. 2). The first two constrained axes accounted for 30% of the variation in the PLFA composition of the samples. Overall, 40% of the variation was constrained. All four of the included variables explained a significant proportion of the variation in the dataset. pH was the factor that accounted for the largest amount of constrained variation, and was orientated roughly parallel to the first constrained axis (RDA1). The effect of salinity on the PLFA composition was orientated along the second constrained axis (RDA2) (Fig. 2). Increasing salinity had an impact on the PLFA profile that was in opposite direction of the effect of the incubation with plant material. PLFAs that were associated with high salinity included 18:1ω9 and x3.

These PLFAs were also more abundant on day 1 of the incubation experiment. PLFAs that were associated with low salinity and had increased in abundance on day 17 included 15:0, a15:0, i15:0, 17:0, 17:1ω8 and 18:1ω9. PLFAs that were associated with high pH included 16:1ω7c, 16:1ω7t, i17:0, 18:0 and 10Me18:0, while 18:2ω6,9 and cy19:0 were associated with low pH.

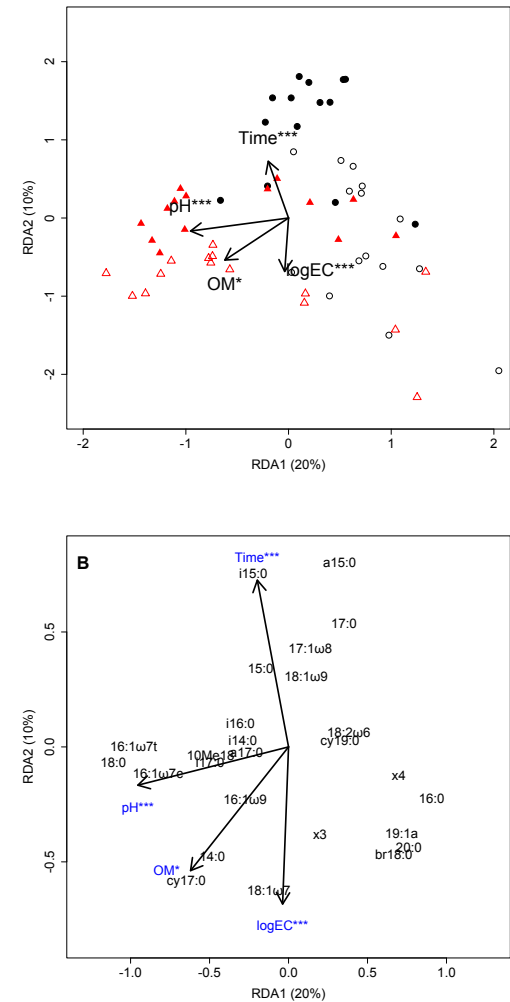**Fig. 2**
(A) Ordination based on an Redundancy analysis (RDA) of the PLFA profile in samples from the AG gradient (black) and the NV gradient (red) from day 1 (empty symbols) and on day 17 (full symbols). Variables used as constraining variables in the RDA are the logarithm of the soil salinity (logEC), pH, soil organic matter content (OM) and time point (day 1 and day 17). Asterisks denote the significance level in a PERMANOVA between the environmental variables and the ordination. (B) Loadings for individual PLFA in the RDA.
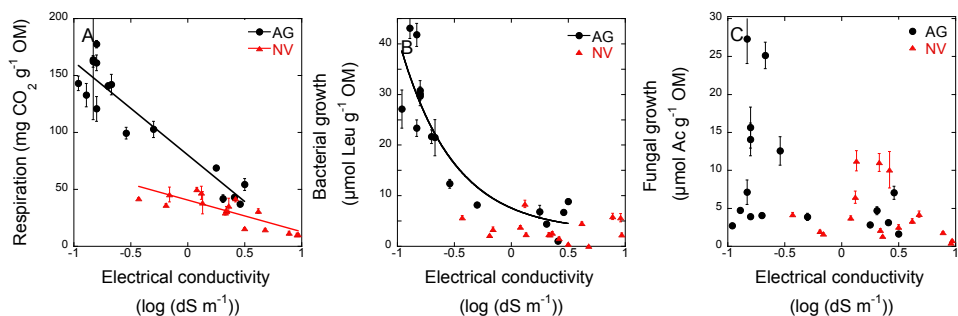
Fig.3
Cumulative respiration (A), cumulative bacterial growth (B) and cumulative fungal growth (C) along the AG and NV salinity gradients over 17 days after addition of plant material to soil.
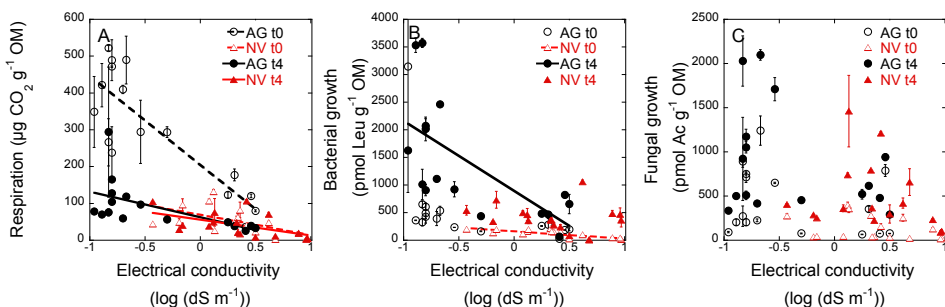

Fig. 4
Respiration (A), bacterial growth (B) and fungal growth (C) along the AG and NV salinity gradients on day 1 (t0) and on day 17 (t4) after addition of plant material to soil. Regression curves show significant relationships between respiration or growth rates and soil salinity.

3.4. Respiration, bacterial and fungal growth

Cumulative respiration over the course of the incubation experiment decreased significantly ($R^2 = 0.87$, $p<0.001$) with increasing salinity along the AG gradient from ca. 150 mg CO_2 produced per g OM in non-saline sites to ca. 50 mg CO_2 g-1 OM (Fig. 3A). A strong decrease of cumulative respiration with increasing salinity was also observed along the NV gradient ($R^2 = 0.67$, $p<0.001$), from ca. 50 mg CO_2 g-1 OM to ca. 10 mg CO_2 g-1 OM. Cumulative bacterial growth decreased exponentially with increasing salinity along the AG gradient ($R^2 = 0.81$) (Fig. 3B). Along the NV gradient there was no relationship between cumulative bacterial growth and salinity. Cumulative fungal growth was not correlated with salinity along either gradient (Fig. 3C).

On day 1, shortly after addition of plant material, soil respiration was negatively correlated with salinity in the AG (R2 = 0.70, p<0.001) and the NV gradient (R2 = 0.30, p<0.05) (Fig. 4A). On day 17 respiration still declined with salinity along both the AG and NV gradients (R2 = 0.30, p<0.05 and R2 = 0.23, p<0.05). Respiration was highest on day 1 after addition of plant material, and gradually declined over time (Fig. S1). While a trend towards a decline in bacterial growth with salinity could be seen in samples from the AG gradient on day 1, this trend was not significant (Fig. 4B). In samples from the NV gradient, a significant negative correlation of bacterial growth with salinity was found (R2 = 0.58, p<0.001). After incubation with plant material on day 17, bacterial growth was negatively correlated with salinity in samples from both the AG (R2 = 0.40, p<0.01) but in the NV gradient the relationship between bacterial growth and salinity disappeared (Fig. 4B). Bacterial growth was highest on day 2 after addition of plant material (Fig. S2). In soils with low salinity bacterial growth increased strongly with the addition of plant material, but in highly saline sites bacterial growth was only marginally boosted. Fungal growth was never significantly correlated with salinity along either gradient (Fig. 4C). Fungal growth was highest around day 4 in less saline samples and around day 7 in highly saline soils (Fig. S3).
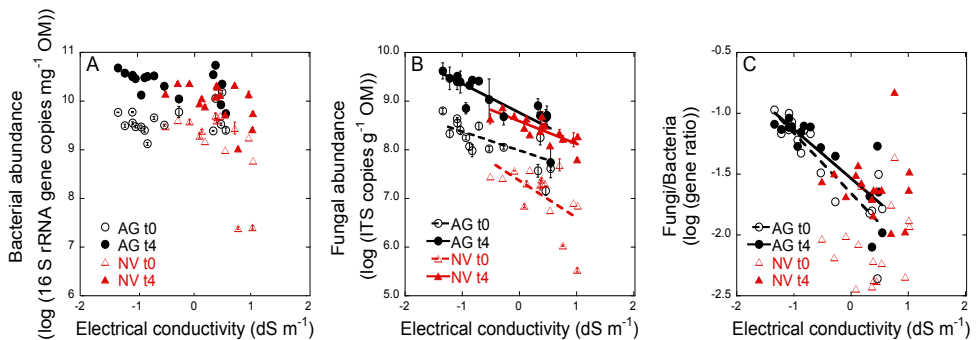

Fig. 5
Abundances of bacterial 16S rRNA genes (A), fungal 18S rRNA genes (B) and the ratio between bacterial/fungal rRNA genes (C) along the AG and NV salinity gradients on day 1 (t0) and on day 17 (t4) after addition of plant material to soil. Regression curves show significant relationships gene copy numbers and soil salinity.

3.5. qPCR

In the incubation experiment with plant material bacterial 16S rRNA gene copy numbers (t = 7.8, p < 0.001) and fungal ITS copy numbers (t = 4.9, p< 0.001) were both significantly higher on day 17 than on day 1 (Fig. 5). Both bacterial 16S rRNA gene and fungal ITS copy numbers increased by about a factor of 5 to 10.

The ratio of fungal/bacterial rRNA genes slightly, but significantly increased during the incubation with plant material between day 1 and day 17 (t= 4.3, p < 0.001). On day 1 the fungal/bacterial ratio ranged from ca. 0.1 to ca. 0.003, while on day 17 it ranged from ca. 0.2 to ca. 0.005.

In the AG gradient bacterial 16S rRNA gene copy numbers at the start of the incubation with plant material were similar in all samples along the gradient at around 1×10^9 to 1×10^{10} copies g-1 OM (Fig. 5). After incubating for 17 days 16S rRNA gene copy numbers ranged from 4×10^9 to 7×10^{10} -g-1 OM. Abundances of fungal 18S rRNA genes ranged from 1×10^7 to 6×10^8 copies g-1 OM on day 1 and from 5×10^7 to 6×10^9 copies mg-1 OM on day 17, and were negatively correlated with salinity both on day 1 ($R^2 = 0.15$, p<0.05) on day 17 ($R^2 = 0.60$, p<0.001). The ratio of fungal/bacterial rRNA gene copy numbers was negatively correlated with salinity on day 1 ($R^2 = 0.69$, p<0.001) and on day 17 ($R^2 = 0.33$, p<0.05).

Along the NV gradient bacterial 16S rRNA gene copy numbers ranged from 2×10^5 to 5×10^9 copies g-1 OM on day 1 and from 9×10^9 to 3×10^{10} copies g-1 OM on day 17 (Fig. 5). Bacterial 16S rRNA gene copy numbers on day 1 and on day 17 were not correlated with salinity. On day 1, fungal ITS copy numbers ranged from 3×10^5 to 5×10^7 copies g-1 OM and were weakly negatively correlated with salinity ($R^2 = 0.19$, p<0.05). On day 17, fungal ITS copy numbers had increased to around 5×10^7 to 8×10^8 copies g-1 OM and were correlated more strongly with salinity ($R^2 = 0.55$, p<0.01), decreasing as salinity increased. The ratio of fungal/bacterial rRNA gene copy numbers was not correlated with salinity in samples from the NV gradient.

4. Discussion

4.1 Salinity effect on biomass, growth and respiration

Based on data collected from previous studies that showed no consistent patterns of biomass with salinity (Rath and Rousk, 2015), we expected biomass to be less impacted by salinity along the gradients than growth and respiration. Overall, biomass measurements based on the abundance of PLFAs were not strongly related to salinity (Fig. 1). Only along the AG gradient was there a relationship between salinity and total and bacterial biomass. Along the NV gradient, which covered a wider span of salinity, biomass concentrations were not related to salinity at any time point and were similar in all samples along the gradient. When the abundance of microorganisms was estimated using q-PCR, fungal ITS copy numbers decreased significantly with increasing salinity, while bacterial 16S rRNA gene copy numbers were not significantly related to salinity and were similar in all samples (Fig. 5).

As expected, both respiration and bacterial growth decreased more strongly with increasing salinity than biomass measurements (Fig. 3, 4). The impact of salinity was stronger along the AG gradient, which also had higher respiration and bacterial growth rates at its non-saline end. Particularly bacterial growth over the course of the incubation with plant material was strongly inhibited in saline soils. Respiration also declined with salinity, which indicates that less of the added plant material was mineralized at high salinities. Fungal growth in the incubation was unaffected by salinity along both gradients (Fig. 3,4), indicating a higher capability of fungi to adapt to salinity and grow on the supplied substrate in saline soils compared to bacteria. The inhibition of respiration by salinity was less pronounced than that of bacterial growth (Fig. 3). The lower inhibition of respiration compared to that of bacterial growth could have been caused by the lack of salt inhibition of fungi, with the fungal contribution offsetting losses in mineralization activity caused by the inhibition of bacteria. No clear relationships emerged between growth rates and respiration on the one hand, and measurements of microbial biomass on the other hand, adding to the growing body of evidence that biomass measurements are not good predictors of process rates (Pausch et al., 2016; Rousk, 2016).

4.2. Microbial responses to plant material additions

Organic matter additions are frequently used amelioration strategies of saline soils (Qadir et al., 2014), primarily because of their beneficial impact on plant growth, but they have also been reported to improve microbial activity in saline soils (Rao and Pathak, 1996; Wichern et al., 2006). Here, we used additions of alfalfa/wheat straw mixture to compensate for differences in organic matter quantity and quality in soils of different salinities, which allowed us to differentiate effects of salinity from those of organic matter content. With the addition of plant material, PLFA concentrations in the soil increased slightly and the relationship between biomass and salinity became weaker (Fig. 1). In contrast to the limited response of total PLFA concentrations in soil, the PLFA composition changed considerably during the incubation with plant material between 1 and 17 days (Fig 2). Many of those PLFAs that increased in relative abundance between 1 and 17 days are the same ones that were associated with low salinity. Over the course of the incubation experiment, organic matter additions thus shifted the PLFA composition towards one more resembling that of a less saline soil. These changes in the PLFA profile could reflect physiological changes in the microbial community that took place during the incubation with additional substrate. Microorganisms are also known to change the PLFA composition of their membranes in response to environmental conditions, such as the availability of nutrients and water (Kieft et al., 1994). However, large differences in microbial community composition between saline

and non-saline soils have been found along the gradients (Rath et al., unpubl.), making it likely that a large proportion of the differences in the PLFA profile are in fact associated with differences in community composition.

Already on the first day after addition, the additional substrate resulted in strongly increased respiration rates (Fig. 4), which gradually declined over time (Fig. S1). However, the increase in growth of both bacteria and fungi lagged behind and increased more slowly after substrate addition (Fig. S2, S3). Initially, added substrate was thus primarily used for respiration rather than for the build-up of new biomass. Between 1 and 17 days of the incubation experiment, the inhibition of respiration by salinity decreased, with smaller differences in respiration between saline and non-saline samples (Fig. 4). The opposite pattern was seen in bacterial growth along the AG gradient, with addition of substrate enhancing differences in bacterial growth between non-saline and saline soils (Fig 4). Plant material in non-saline soils led to a large increase in bacterial growth, whereas in saline soils bacterial growth rates were only slightly increased. In contrast, along the NV gradient the inhibition of bacterial growth by salinity was alleviated by addition of organic matter, although bacterial growth rates remained comparatively low in soils of all salinities (Fig. 4). Addition of plant material also increased fungal growth, but the duration for fungal growth to reach its maximum rate happened more slowly than for bacterial growth, suggesting that bacteria were able to more quickly use the added plant material in soils with low salinity (Fig. S2, S3). In general, fungal growth responded faster to substrate additions in non-saline soils than in saline soils (Fig. S3), but overall, fungal growth after substrate additions was similar in soils of all salinities with no clear differences between soils of low and high salinity. A beneficial effect of substrate addition in saline soils could be seen for both microbial growth and respiration, which confirms the positive effect of organic matter amendments on the microbial activity in saline soils. Despite the positive effect of substrate additions, respiration and bacterial growth remained reduced in saline soils compared to non-saline soils, which indicates a direct effect of salinity that cannot be compensated for completely by increased supply of substrate.

Compared to the slight increase in PLFA concentrations following addition of plant material (Fig. 1), copy numbers of rRNA genes increased more strongly both for fungal and bacterial markers (Fig. 5). Why PLFA concentrations overall increased less than gene copy numbers in response to substrate addition is unclear. DNA has been suggested to have a much faster turnover in soil than PLFA (Malik et al., 2015), which means that DNA-based biomass measurements could reflect more recently produced biomass than PLFA-based measurements. Since PLFA-based measurements of biomass contain a larger proportion of necromass, increases in total biomass by newly produced biomass would thus be more

apparent, forming a higher fraction of the total, in DNA-based measurements than in PLFA-based measurements.

The increase in rRNA gene copy numbers was larger for fungal ITS copy numbers than for bacterial 16S rRNA gene copy numbers, suggesting a larger increase in fungal abundance during incubation with plant material (Fig. 5). Consequently the ratio of fungal/bacterial rRNA gene copies increased from day 1 to day 17 after addition of plant material. The addition of plant material thus appeared to promote build-up of fungal biomass over the build-up of bacterial biomass.

4.3. Differential effect of salinity on bacteria and fungi

Based on prior findings of increased fungal resistance to acute salt exposure in soil (Rath et al., 2016), we expected fungi to be less affected by salinity along the studied natural salinity gradients. In accordance with our hypothesis, along the AG gradient, fungal growth was less inhibited by salinity than bacterial growth (Fig. 3). However, along the NV gradient, after the initial inhibition of bacterial growth was overcome by incubation with plant material, the two decomposer groups did not clearly differ in their performance along the gradient. In any case, based on microbial growth rate we see no indication that the general view that saline habitats would be more prokaryote-dominated (Horikoshi and Grant, 1998) should hold true for saline soils.

The two methods used to estimate biomass provided contradictory information on the relationship between bacterial and fungal abundance and salinity (Fig. 1, 5). While the fungal biomarker PLFA 18:2ω6,9 did not change in abundance along the salinity gradients, fungal ITS gene copy numbers decreased significantly with increasing salinity. In contrast, whereas bacterial PLFAs were negatively correlated with salinity, bacterial abundance of 16S rRNA genes did not show a relationship with salinity. Use of different methods would thus lead us to contrasting conclusions about shifts in fungal/bacterial ratio in the study systems at hand. Both PLFA biomarker-based as well as qPCR-based assessments of biomass bring with them their own biases related to shifts in PLFA content and composition of membranes as well as cellular rRNA gene contents with microbial physiology and community composition. Ultimately, whether the ratio of fungal/bacterial biomass was negatively or positively related to salinity could not be resolved conclusively using proxy measurements for biomass.

Conclusions

Along the two gradients of salinity included in this study we saw a negative impact of salinity on microbial biomass, respiration and bacterial growth. Biomass

measurements were less strongly affected by salinity than either respiration or bacterial growth, and were not clearly related to either process. We conclude therefore that the use of biomass as a proxy to infer the impact of environmental conditions on microbial process rates in soil is insufficient. Organic matter additions alleviated some of the negative impact on the microbial community along the salinity gradients. However, respiration and bacterial growth remained strongly negatively affected by salinity, identifying a direct effect of salinity on those processes. Based on microbial growth rate measurements fungi were less negatively affected by salinity than bacteria, suggesting that fungi could be of higher importance in salt-affected soils than generally believed.

Acknowledgements

References

Bååth, E., 1994. Thymidine and leucine incorporation in soil bacteria with different cell size. Microbial Ecology 27, 267-278.

Bååth, E., 2001. Estimation of fungal growth rates in soil using C-14-acetate incorporation into ergosterol. Soil Biology & Biochemistry 33, 2011-2018.

Bååth, E., Pettersson, M., Söderberg, K.H., 2001. Adaptation of a rapid and economical microcentrifugation method to measure thymidine and leucine incorporation by soil bacteria. Soil Biology and Biochemistry 33, 1571-1574.

Chowdhury, N., Marschner, P., Burns, R., 2011. Response of microbial activity and community structure to decreasing soil osmotic and matric potential. Plant and Soil 344, 241-254.

Fierer, N., Jackson, J.A., Vilgalys, R., Jackson, R.B., 2005. Assessment of soil microbial community structure by use of taxon-specific quantitative PCR assays. Applied and Environmental Microbiology 71, 4117-4120.

Frostegård, A., Bååth, E., 1996. The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. Biology and Fertility of Soils 22, 59-65.

Grant, W.D., 2004. Life at low water activity. Philosophical Transactions of the Royal Society of London Series B-Biological Sciences 359, 1249-1266.

Horikoshi, K.k., Grant, W.D., 1998. Extremophiles : microbial life in extreme environments. Wiley-Liss, New York.

Kamble, P.N., Gaikwad, V.B., Kuchekar, S.R., Bååth, E., 2014. Microbial growth, biomass, community structure and nutrient limitation in high pH and salinity soils from Pravaranagar (India). European Journal of Soil Biology 65, 87-95.

Kieft, T.L., Ringelberg, D.B., White, D.C., 1994. Changes in Ester-Linked Phospholipid Fatty-Acid Profiles of Subsurface Bacteria during Starvation and Desiccation in a Porous-Medium. Applied and Environmental Microbiology 60, 3292-3299.

Klappenbach, J.A., Dunbar, J.M., Schmidt, T.M., 2000. RRNA operon copy number reflects ecological strategies of bacteria. Applied and Environmental Microbiology 66, 1328-1333.

Malik, A.A., Dannert, H., Griffiths, R.I., Thomson, B.C., Gleixner, G., 2015. Rhizosphere bacterial carbon turnover is higher in nucleic acids than membrane lipids: implications for understanding soil carbon cycling. Frontiers in Microbiology 6.

Pausch, J., Kramer, S., Scharroba, A., Scheunemann, N., Butenschoen, O., Kandeler, E., Marhan, S., Riederer, M., Scheu, S., Kuzyakov, Y., Ruess, L., 2016. Small but active - pool size does not matter for carbon incorporation in below-ground food webs. Functional Ecology 30, 479-489.

Qadir, M., Quillerou, E., Nangia, V., Murtaza, G., Singh, M., Thomas, R.J., Drechsel, P., Noble, A.D., 2014. Economics of salt-induced land degradation and restoration. Natural Resources Forum 38, 282-295.

Rao, D.L.N., Pathak, H., 1996. Ameliorative influence of organic matter on biological activity of salt-affected soils. Arid Soil Research and Rehabilitation 10, 311-319.

Rath, K.M., Maheshwari, A., Bengtson, P., Rousk, J., 2016. Comparative toxicity of salts to microbial processes in soil. Applied and Environmental Microbiology, AEM. 04052-04015.

Rath, K.M., Rousk, J., 2015. Salt effects on the soil microbial decomposer community and their role in organic carbon cycling: A review. Soil Biology & Biochemistry 81, 108-123.

Rengasamy, P., 2006. World salinization with emphasis on Australia. Journal of Experimental Botany 57, 1017-1023.

Richards, L.A., 1954. Diagnosis and improvement of saline and alkali soils. Soil Science 78, 154.

Rousk, J., 2016. Biomass or growth? How to measure soil food webs to understand structure and function. Soil Biology & Biochemistry 102, 45-47.

Sardinha, M., Muller, T., Schmeisky, H., Joergensen, R.G., 2003. Microbial performance in soils along a salinity gradient under acidic conditions. Applied Soil Ecology 23, 237-244.

Six, J., Frey, S.D., Thiet, R.K., Batten, K.M., 2006. Bacterial and fungal contributions to carbon sequestration in agroecosystems. Soil Science Society of America Journal 70, 555-569.

Strickland, M.S., Rousk, J., 2010. Considering fungal:bacterial dominance in soils - Methods, controls, and ecosystem implications. Soil Biology & Biochemistry 42, 1385-1395.

Szabolcs, I., 1989. Salt-Affected Soils. C R C Press LLC.

Wichern, J., Wichern, F., Joergensen, R.G., 2006. Impact of salinity on soil microbial communities and the decomposition of maize in acidic soils. Geoderma 137, 100-108.

Yuan, B.C., Xu, X.G., Li, Z.Z., Gao, T.P., Gao, M., Fan, X.W., Deng, H.M., 2007. Microbial biomass and activity in alkalized magnesic soils under arid conditions. Soil Biology & Biochemistry 39, 3004-3013.
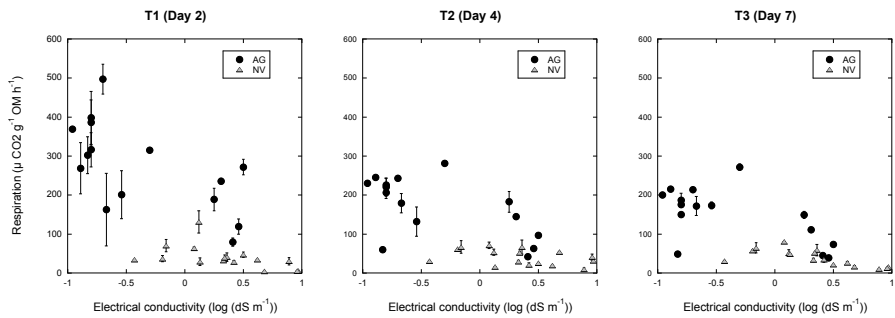
Supplemental information

Fig. S1: Respiration per g organic matter in samples of different salinities from the AG and NV gradient on day 2, 4 and 7 after addition of plant material.
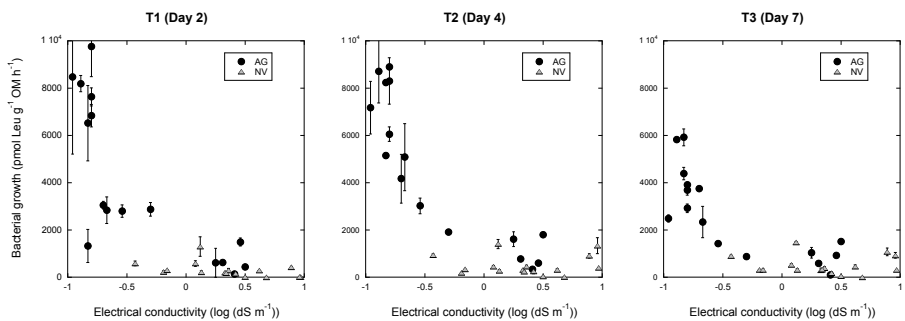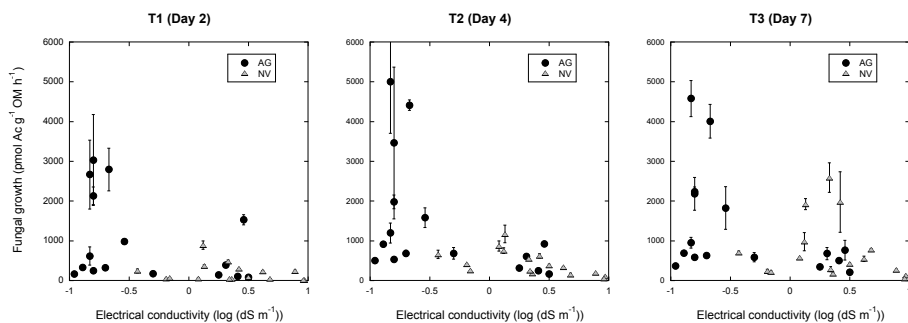

Fig. S2: Bacterial growth per g organic matter in samples of different salinities from the AG and NV gradient on day 2, 4 and 7 after addition of plant material.

Fig. S3: Fungal growth per g organic matter in samples of different salinities from the AG and NV gradient on day 2, 4 and 7 after addition of plant material.

The impact of salinity on the microbial response to drying and rewetting in soil

Kristin M. Rath [a, b, *], Arpita Maheshwari [a], Johannes Rousk [a]

[a] Section of Microbial Ecology, Dept. of Biology, Ecology Building, Lund University, Lund, Sweden
[b] Centre for Environmental and Climate Research (CEC), Lund University, Lund, Sweden

ABSTRACT

In saline soils, the severity of drought for the soil microbial community is exacerbated by accumulating concentrations of salts during drying. In this study we investigated how bacterial growth and respiration responses to drying-rewetting were affected by salinity. To do this, we adjusted a non-saline soil to four different salinities (0, 2, 7 and 22 mg NaCl g^{-1}), followed by addition of plant material and a one-month incubation. Following the incubation period, we assessed the moisture dependence of respiration and growth, as well as the responses of bacterial growth and respiration to a cycle of air-drying followed by rewetting to optimal moisture. The inhibition of bacterial growth and respiration by reducing moisture increased with higher salt concentrations. As such, salinity was shown to increase the negative impact of drying on bacterial growth and alter the bacterial growth and respiration dynamics after rewetting. Drying-rewetting of soils with low salinity resulted in an immediate onset and gradual resuscitation of bacterial growth to levels similar to before drying. In contrast, in soils with higher salinity growth increased exponentially after a lag period of several hours. The duration of the lag period induced by salinity increased with the amount of salt added. The observed lag period matched previously reported results observed in response to more severe drying by e.g. longer duration of drought and drought combined with starvation. In treatments with a salt concentration ≤7 mg NaCl g^{-1} a high respiration pulse occurred immediately after rewetting and subsequently respiration declined. In the most saline treatment the initial respiration was reduced below the level of continuously moist soil to later increase exponentially in parallel with the increase in bacterial growth. We conclude that soil salinity increases the inhibition of microbial activity by low moisture, that fundamentally different responses to drying-rewetting cycles can be induced, and that high salt concentrations can substantially delay the pulse of respiration induced by rewetting dry soil.

1. Introduction

Rewetting dried soil generally results in one of two types of responses of respiration and microbial growth (Fig. 1), during which respiration and bacterial growth rates have been found to be uncoupled (Meisner et al., 2013). In the first type of response (henceforth "type 1 response") a linear increase in growth rate starts immediately after rewetting, recovering growth rates to levels similar to those before drying within hours. This coincides with a respiration rate that is highest immediately after rewetting and subsequently decreases exponentially towards rates similar to before drying within days (Fig. 1). In the second type of response (henceforth "type 2 response"), an initial lag period of almost no growth occurs, lasting for up to 20 h, followed by an exponential increase of growth to levels far exceeding those before drying. This response coincides with elevated respiration rates that remain high for an extended duration and that are sometimes followed by a secondary increase that occurs simultaneously with the exponential increase in growth (Fig. 1). Whether the growth response to drying and rewetting in a particular soil follows a type 1 or a type 2 response has been proposed to be influenced by the severity of drying. For instance, a lag period in growth rates after rewetting can be induced by increasing the duration of drought before rewetting, as well as by a prolonged period of storage of soil samples prior
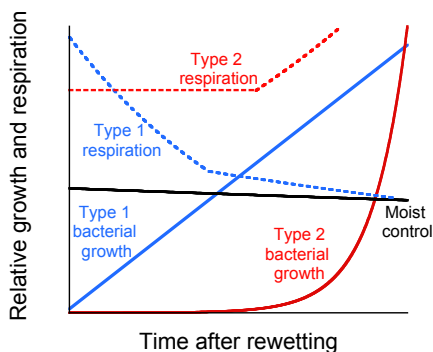
Fig. 1. Schematic illustration of type 1 (blue) and type 2 (red) responses of bacterial growth (solid line) and respiration (dashed line) to drying-rewetting. A type 1 response after drying-rewetting is characterized by a linear increase in bacterial growth, accompanied by an initial high respiration pulse that decreases exponentially over time. A type 2 response is characterized by a lag period of almost no growth together with elevated respiration rates, followed by an exponential increase in growth rate which coincides with an extended duration of elevated respiration rates and sometimes a secondary increase in respiration. The black line marks the continuously moist control for both bacterial growth and respiration, which decrease slightly over the course of the experimental period. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

to drying-rewetting which is thought to induce starvation (Meisner et al., 2015).

During drying of soil, the concentration of ions in the soil solution increases. High salt concentrations in soils are known to negatively impact microbial activity (Rath and Rousk, 2015). The combination of low soil water content and high salt concentrations could interact and exacerbate the negative effect of the individual factors on the soil microbial community. Even though saline soils are widespread in arid and semi-arid areas of the world, where droughts are a common occurrence, to date the effects of drying and rewetting (drying-rewetting) on the microbial community have been mainly studied in non-saline soils. In fact, only a handful of studies have looked at the combined effect of low water content and high salinity on the response of respiration and microbial growth to drying-rewetting (Chowdhury et al., 2011a, 2011b; Mavi and Marschner, 2012; Kakumanu and Williams, 2014).

This study consisted of a two-part experiment. Microcosms were set up by adjusting a non-saline soil to different salinities through addition of different amounts of NaCl. The microcosms were also supplied with additions of plant material, in order to provide the microbial communities with additional resources to fuel adaptation to salinity. First, we dried soils of different salinities and monitored the moisture dependences of respiration and bacterial growth during drying. Second, we rewetted dried soils of different salinities to study the influence of salinity on the dynamics of microbial growth and respiration after rewetting. We hypothesized (i) that the severity of drying experienced by the soil microbial community would increase with salinity (H1), and (ii) that higher severity of drying in the more saline soils would induce a shift from a type 1 response to a type 2 response of bacterial growth and respiration (H2).

2. Material and methods

2.1. Soil

Soil was collected in May and June 2015 from a permanent grassland site in Vomb, south Sweden (55° 40′ 27″ N, 13° 32′ 45″ E).

The soil is a well-drained sandy grassland soil and classified as a Eutric Cambisol (IUSS Working Group WRB, 2006) or Inceptisol (Soil Survey Staff, 1999). Multiple soil samples were collected with a spade from pits dug to a depth of ca. 20 cm and combined into composite samples, homogenized, and sieved (<2.8 mm). The soil had a water content (gravimetric, 24 h at 105 °C) of ca. $28 \pm 0.4\%$ dry weight (dw) (mean $\pm$ 1 SE of three replicates), a water holding capacity (WHC) of $65 \pm 2\%$ dw and an organic matter content (loss on ignition, 600 °C for 12 h) of $19.6 \pm 0.6\%$ dw. In a 1:5 soil:water mixture the pH was 6.1 ± 0.02 and the electrical conductivity was 0.09 ± 0.005 dS m^{-1}. Soil properties did not differ between soil sampled in May and June. Experiment 1 was performed on soil sampled in May only, whereas experiment 2 was performed on soil samples collected in both May and June.

2.2. Microcosm setup

Soil (250 g) was weighed into 1-l plastic containers with airtight lids and adjusted to four different salinity levels through the addition of different amounts of NaCl (0, 2.5, 7.3 and 22.2 mg NaCl g^{-1} soil) together with 100 μl of H$_2$O per g soil. These salt additions resulted in a salt concentration of 0, 12, 31 and 98 mg NaCl per g H$_2$O in moist soil. Electrical conductivity in the four treatments, measured in a 1:5 soil:water mixture (EC$_{1:5}$), was 0.1, 1.1, 2.8 and 6.8 dS m^{-1}. This corresponds to an electrical conductivity in saturated paste ranging from ca. 1—90 dS m^{-1} (Rengasamy, 2006). For each level of salinity three replicate microcosms were set up. A complete set of 12 microcosms was set up with each of the soil samples collected in May and June. The soils were then incubated in the dark for 3 weeks at 18 °C with 15 mg 1:1 wheat straw —alfalfa g^{-1} soil that was mixed into the soil by prolonged shaking on a vortex mixer. The added plant material had a C content of ca. 45% and a C/N ratio of ca. 45 (Dumas dry combustion, VarioMAX CN, Elementar, Hanau Germany). The particle size of the ground and sieved plant material was between 250 and 630 μm. Previously it was found that straw with a high C/N ratio predominantly stimulated fungal growth, whereas alfalafa with a lower C/N ratio predominantly stimulated bacterial growth (Rousk and Bååth, 2007). A mixture of both straw and alfalfa should therefore stimulate both fungal and bacterial growth. A water content of ca. 60% WHC was maintained throughout the incubation period and microcosms were regularly aired and mixed to prevent anoxic conditions. Previous experiments showed that three weeks incubation time after salt addition was a sufficiently long time period for the community to adapt to the increased salt concentrations, as was shown by an induced community tolerance occurring within ca. 1 week following salt exposure (Maheshwari, 2015).

2.3. Experiment 1: direct moisture dependence of respiration and bacterial growth

After an incubation period of three weeks at different salinities (see above) a subset of soil from each microcosm was gradually air-dried at 23 °C under a fan over a period of 3—4 days. Water content was monitored by weighing of microcosms and once a target soil water content was reached, subsamples of soil were collected and stored at 5 °C in closed vials for later analysis of respiration and microbial growth rates (see section 2.5.). The drying continued until the microcosms reached a constant weight, i.e. the soil was completely air-dried at a water content of ca. 1% WHC. The selected target soil water contents covered a range of soil water contents from moist soil to completely air-dried soil. All samples were analyzed simultaneously after the end of the experiment.

2.4. Experiment 2: rewetting responses

After an incubation period of one month at different salinities (see above), a subset of soil of each replicate microcosm was completely air-dried for 3–4 days at 23 °C under a fan until moisture stabilized, resulting in a water content of ca. 1% WHC. The drying procedure was the same in both experiments 1 and 2. The reduced water content that resulted after drying increased the nominal concentration of NaCl in the pore water to 0, 65, 180 and 405 mg g^{-1} remaining H$_2$O. Following these four days, the soil was rewetted to a water content of 60% WHC, and responses of respiration and bacterial growth (see section 2.5.) were monitored over a time period of approximately 50–70 h. Another subset of soil was kept continuously moist at 60% WHC throughout the experiment. For both the soil samples collected in May and June, the experiment was repeated in full at the same water content of 60% WHC. The second sample in June was taken to enable repetition of the experiment on a fresh soil sample.

2.5. Measurements

2.5.1. Respiration

Soil (1 g) was weighed into a 20 ml glass vial. The head-space was purged with pressurized air, sealed and incubated at 18 °C in the dark for ca. 4 h. Afterwards the CO$_2$ concentration in the headspace was analysed using a gas chromatograph (GC), equipped with a methanizer and a flame ionization detector. Background levels of CO$_2$ in pressurized air were subtracted.

2.5.2. Bacterial growth

The bacterial growth rate was estimated by measuring the incorporation of ^{3}H-labelled leucine (Leu) into bacteria extracted from soil according to Bååth (1994) and Bååth et al. (2001). Soil (1 g) was mixed with 20 ml of water followed by a 10 min centrifugation step at 1000 g. From the resulting bacterial suspension a 1.5 ml subsample was used to measure bacterial growth. Tritiated leucine (2 μl [^{3}H] Leu, 37 MBq ml^{-1}, 5.74 TBq mmol^{-1}; Perkin Elmer, UK) was added to the suspension together with non-labelled Leu, resulting in a final concentration of 275 nM Leu. After a 1 h incubation at 18 °C in the dark bacterial growth was terminated by the addition of 100% trichloroacetic acid. After a series of washing steps (Bååth et al., 2001) the amount of incorporated radioactive label was measured using liquid scintillation.

2.5.3. Determination of biomass using phospholipid fatty acid (PLFA) analysis

At the end of the initial three week incubation period before the beginning of experiment 1 phospholipid fatty acids (PLFAs) were extracted and analyzed using a procedure described by Frostegård et al. (1993). Briefly, soil was extracted in a single-phase mixture of chloroform:methanol:citrate buffer (1:2:0.8 v/v/v). After extraction the phospholipids were separated from other lipids on a silicic acid column. The phospholipids were methylated and separated on a gas chromatograph equipped with a flame ionization detector. Peak areas were quantified by adding methyl nonadecanoate fatty acid (19:0) as the internal standard before the methylation step. The sum of the following PLFAs was used a measure of the bacterial biomass: i14:0, i15:0, a15:0, 15:0, i16:0, 10Me16:0, i17:0, a17:0, cy17:0, 17:0, br18:0, 10Me17:0, 18:1ω7, 10Me18:0 and cy19:0, while 18:2ω6,9 was used as a measure of fungal biomass (Frostegård and Bååth, 1996).

2.6. Data analysis

The amounts of total, bacterial and fungal PLFAs between different salinity treatments were compared using ANOVA. The 26 most abundant PLFAs (Fig. 2b) were converted to relative abundances (mol%) and the PLFA composition was then analyzed with a principal component analysis (PCA), after standardizing to unit variance. ANOVA followed by Tukey's HSD test were used to test for significant differences between treatments along principal components 1 (PC1) and 2 (PC2) ($\alpha = 0.05$).

For both experiments, microbial growth rate and respiration were calculated per dry mass of soil. In experiment 1, microbial growth rates and respiration in samples of different moisture were normalized to the mean of the values measured in the moist soil (Table S1). The moisture dependence of growth and respiration was modeled with a logistic model using the following equation:

$$y = \frac{c}{(1 + e^{-b(x-a)})}$$

where y is the process rate, x is the soil moisture, a is the soil moisture at which the measured process is inhibited by 50% (IC$_{50}$), c is the process rate in the control and b is a parameter indicating the inhibition rate. The same equation was also used to model the inhibition of microbial processes with increasing salt concentration in the soil solution, with the logarithm of the salt concentration in the remaining soil water replacing soil moisture (Fig. S2). To see if a parameter that captures both the effects of low soil moisture and salinity could predict the response of respiration and bacterial growth, we modeled total water potential in the soil. To estimate the effect of decreasing soil moisture, we used water potentials in a typical loam soil (Brady and Weil, 2002) to which osmotic potentials achieved in the soil solution during drying in the salt treatments were added. Osmotic potential O_s (MPa) in the soil solution at each moisture content was estimated based on the relationship described in Richards (1954):

$$O_s = -0.036 \, EC_{1:5} \left(\frac{\theta_{ref}}{\theta_{act}} \right)$$

where $EC_{1:5}$ is the electrical conductivity in a 1:5 soil:water mixture in dS m^{-1}, θ_{ref} is the reference water content at which the electrical conductivity was measured (in this case 5 ml g^{-1}), and θ_{act} is the actual water content of the soil in g H$_2$O g^{-1}. Soil water potentials were converted to positive values and log-transformed. The relationship between water potential, and respiration and growth was modeled with the same logistic model used for soil moisture dependence, with water potential replacing soil moisture (Fig. S3).

For experiment 2, the data obtained from the two repetitions of the experiment were combined. Bacterial growth and respiration data from the drying-rewetting treatments were normalized to the mean of the rates measured in the continuously moist control treatments. Salt treatments with a lag period for bacterial growth after rewetting (7 and 22 mg NaCl g^{-1} treatments) were modeled with a Gompertz growth model until the peak rate for growth was reached. The Gompertz function uses the following model (Gibson et al., 1988; Meisner et al., 2015):

$$G_t = 10^{G_{t0}+A \times e^{-e^{b-ct}}}$$

where G_t is bacterial growth at time t, G_{t0} is bacterial growth at t_0, A is the difference between the upper and lower asymptotes of the curve and b and c are fitted mathematical parameters. The lag period in the onset of bacterial growth after rewetting was then calculated as:

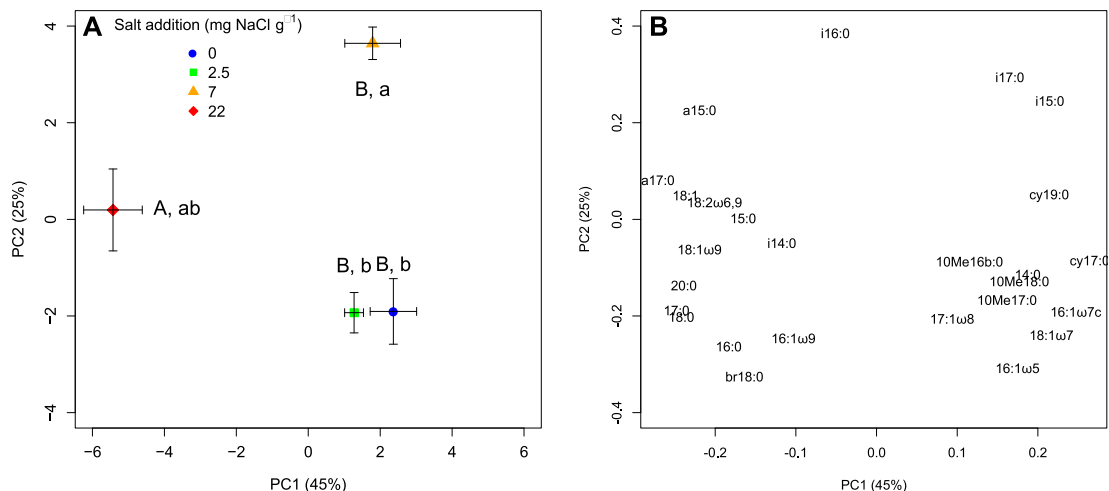$$Lag \; period = \frac{b-1}{c}$$

Fig. 2. Principal Component Analysis (PCA) of the phospholipid fatty acid (PLFA) composition in microcosms of different salinity treatments. Panel A shows the average scores for each treatment for principal components 1 (PC1) and 2 (PC2). Error bars represent the standard error (n = 2). Along PC1 upper case letters indicate significant differences between treatments according to Tukey's HSD test (α = 0.05). Lower case letters indicate significant differences between treatments along PC2. Panel B shows the loadings for individual PLFA in the PCA.

The growth in treatments without a lag period (0 and 2 mg NaCl g^{-1} treatments), and in the 7 and 22 mg NaCl g^{-1} treatments after the peak in growth was reached, was described with a smoothing function. These curves were drawn using the locally weighted least squared error (Lowess) method with a 50% smoothing factor.

A Gompertz model was used to model respiration in the 22 mg NaCl g^{-1} treatment until the maximum respiration rate was reached. Respiration rates in the 0–7 mg NaCl g^{-1} treatments, and in the 22 mg NaCl g^{-1} after the peak in respiration were also described with a smoothing function with a 50% smoothing factor, The software used to model curves was Kaleidagraph 4.5.0 (Synergy Software, Reading, PA, USA).

Cumulative respiration and growth rates over the course of the experiment were calculated for both the drying-rewetting treatment and the continuously moist control treatment. Growth and respiration rates were overall higher in the sample collected in May than the sample from June. Therefore, to compare the influence of salt on continuously moist control rates for both repetitions of the experiment, all cumulative data in the continuously moist control were normalized to the mean of the cumulative respiration and growth rate in the continuously moist control treatment without added salt ca. 50 h after rewetting. To compare the effect of drying-rewetting on soil at each salinity, cumulative data in the drying-rewetting treatments were normalized to the mean of the continuously moist control treatments with the same salt addition.

3. Results

3.1. Microbial biomass and composition

The amount of microbial biomass, measured as the amount of PLFA, did not differ significantly between treatments, although there was a tendency towards lower biomass in the 22 mg NaCl g^{-1} treatment (ca. 100 nmol PLFA g^{-1} dw) than in the other treatments (ca. 140 nmol PLFA g^{-1} dw) (Table 1). The ratio of fungal to bacterial PLFA was similar in all treatments and ranged from 0.11 to 0.15 (Table 1).

PC1 and PC2 combined explained ca. 70% of the variation between samples, of which PC1 explained 45% and PC2 25% (Fig. 2). PC1 was weakly negatively correlated with salt addition to the soil ($R^2 = 0.89$, $p < 0.05$) (Fig. S1). There was no significant correlation between salt addition and PC2. The PLFAs driving the community composition along PC1 towards higher salinity included 18:0, 18:2, 20:0, 18:1ω9, the bacterial PLFAs a17:0 and 17:0, and the fungal PLFA 18:2ω6,9 (Fig. 2b). Meanwhile, increased relative abundance of 16:1ω7c and the bacterial PLFAs i15:0, cy17:0, cy19:0 and 18:1ω7 was associated with lower salinity (Fig. 2b). Samples of the same treatment clustered closely together (Fig. 2a). Samples of the 0 and 2.5 mg NaCl g^{-1} treatments had a similar PLFA composition along both PC1 and PC2 and were grouped closely together in the PCA plot (Fig. 2a). Along PC1 scores of the 22 mg NaCl g^{-1} treatment differed significantly from the other treatments. Along PC2 scores of the 7 mg g^{-1} treatment were significantly different from the 0 and 2.5 mg NaCl g^{-1} treatments.

3.2. Moisture dependence of microbial growth and respiration

The effect of drying at different salinities was compared using three different parameters related to either soil moisture or salinity: soil moisture (in %WHC) (Fig. 3), NaCl concentration in the remaining soil water (Fig. S2), and water potential (Fig. S3). Using only soil moisture as the independent variable, the effect of salinity during drying becomes apparent through differences between salt treatments. On the other hand total water potential and increasing NaCl concentration during drying are independent variables that capture the effect of both decreasing soil moisture during drying and accumulating concentration of NaCl in the remaining soil water.

The moisture dependence of both respiration and bacterial growth could be described well with a logistic model (with R^2 values ranging from 0.81 to 0.98). Respiration decreased at a similar rate with decreasing soil moisture in all salt treatments (Fig. 3a). The IC_{50} (soil moisture at which respiration is inhibited by 50% compared to moist soil) for respiration ranged between 24% and

Table 1

Total, bacterial and fungal PLFA in the four salinity treatments. The following PLFAs were used to calculate the amount of bacterial PLFA: i14:0, i15:0, a15:0, 15:0, i16:0, 10Me16:0, i17:0, a17:0, cy17:0, 17:0, br18:0, 10Me17:0, 18:1ω7, 10Me18:0 and cy19:0. The PLFA 18:2ω6,9 was used as a measure of fungal biomass.

Treatments (mg NaCl g^{-1})	Total PLFA (nmol g^{-1} dw)	Bacterial PLFA (nmol g^{-1} dw)	Fungal PLFA (nmol g^{-1} dw)	Fungal/bacterial PLFA
22	96.5 (2.8)	60.0 (3.7)	9.0 (0.9)	0.15 (0.02)
7	146.1 (12.9)	92.0 (8.0)	12.0 (0.7)	0.13 (0.003)
2.5	134.9 (4.6)	78.3 (5.4)	11.6 (0.4)	0.15 (0.01)
0	140.1 (24.9)	80.2 (12.3)	8.8 (0.3)	0.11 (0.01)

Values represent the mean (n = 2) with 1 standard error in parentheses.

32% WHC. In contrast, bacterial growth rates were inhibited to a higher degree when soil moisture decreased in the more saline treatments than in the soils without added salt (Fig. 3b). While the IC$_{50}$ for bacterial growth in the 0 and 2 mg NaCl g^{-1} treatment was ca. 8% WHC, it increased to 18% in the 7 mg NaCl g^{-1} treatment and 36% in the 22 mg NaCl g^{-1} treatment. As soils dried out, the concentration of the added NaCl in the remaining water in the soil increased. When respiration and growth rates were modeled against the NaCl concentration in the remaining soil water, treatments with a lower salt addition were more sensitive to accumulating NaCl concentrations than treatments that had received higher salt additions at the beginning of the incubation period (Fig. S2). The relationships of both growth and respiration to the logarithm of the water potential could also be described with a logistic model (Fig. S3). The IC$_{50}$ for respiration increased successively from a water potential of −1 MPa in the 0 mg NaCl g^{-1} treatment to −4 MPa in the 2 mg NaCl g^{-1}, -7 MPa in the 7 mg NaCl

g^{-1} and -17 MPa in the 22 mg NaCl g^{-1} treatment (Fig. S3). The IC$_{50}$ of bacterial growth was at water potentials of −19 MPa in the 0 mg NaCl g^{-1} treatment, −13 MPa in the 2 mg NaCl g^{-1} treatment and around −34 MPa in both the 7 and 22 mg NaCl g^{-1} treatments (Fig. S3).

3.3. Bacterial growth responses to drying-rewetting

After rewetting dried soil, growth rates were initially low (Fig. 4). In the 0, 2 and 7 mg NaCl g^{-1} treatments it took ca. 5 h until the growth rate recovered to approximately the rate in the continuously moist control, whereas in the 22 mg NaCl g^{-1} treatment it took ca. 20 h for the growth rate to recover to the same level as in the continuously moist control (Figs. 4 and 5c). The control treatment and the treatment receiving 2 mg NaCl g^{-1} both showed a linear increase in growth rate with time that started immediately after rewetting of soil (Fig. 4), consistent with a type 1 response (Fig. 1). The highest growth rate was reached around 10 h after rewetting. The treatment without salt thereafter maintained a growth rate that was about 1.5 times higher than that in the continuously moist control until the end of the experiment (Fig. 4). In the 2 mg NaCl g^{-1} treatment the growth rate peaked at a rate that was around 5 times higher than continuously moist control and then decreased back to a level that was around 1.5 times that of the continuously moist control (Fig. 4a). The 7 mg NaCl g^{-1} treatment started growing exponentially after a short lag period of 0.6 h and reached a maximum growth rate around 10 h after rewetting (Figs. 4 and 5a). The most saline treatment receiving 22 mg NaCl per g soil showed a pronounced lag period of ca. 15 h with almost no growth, after which growth rates increased exponentially until a maximum was reached ca. 30 h after rewetting (Figs. 4 and 5a). The maximum growth rate induced by drying-rewetting increased with higher salinity (Fig. 5b), from twice the rate of the continuously moist control in the 0 mg NaCl g^{-1} treatment to 9 times higher than the continuously moist control in the treatment receiving 22 mg NaCl g^{-1}.

In continuously moist control samples cumulative growth declined with increasing salinity (Fig. 6a). At the end of the experiment, cumulative growth in drying-rewetting samples was approximately 1.5—4 times higher than the continuously moist control in samples from May, and 1.2 to 3 times higher in samples from June (Fig. 6B). Higher salinity increased the difference between continuously moist control and drying-rewetting, with the highest increase in cumulative growth in drying-rewetting treatments compared to continuously moist control treatments found in the 22 mg NaCl g^{-1} treatment (Fig. 6b).

3.4. Respiration responses to drying-rewetting

In treatments with 0—7 mg NaCl g^{-1} respiration rates were highest shortly after rewetting (Fig. 7) and then decreased exponentially until the end of the experiment. In the 22 mg NaCl g^{-1} treatment respiration rates were low immediately after rewetting and remained suppressed for 5 h (Figs. 7 and 8a). Only then did
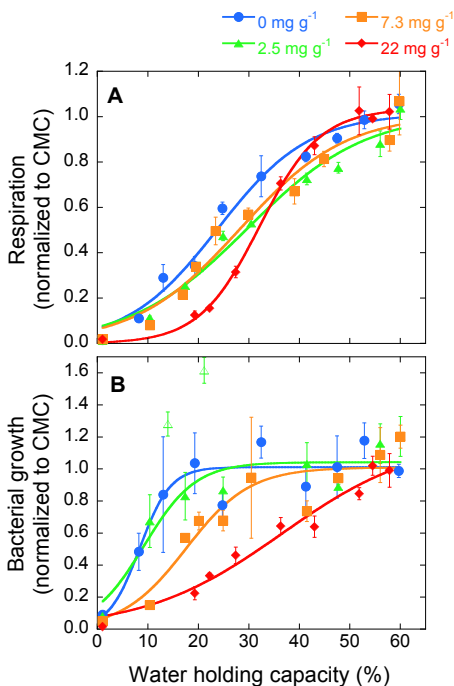

Fig. 3. Logistic inhibition curves of respiration (A), and bacterial growth (B) during drying of soil from a water content of ca. 60% WHC to completely air-dried (R^2 = 0.85 to 0.97). Relative process rates have been normalized to measurements in the continuously moist control soil (CMC) at each salinity level. Data points show the mean (n = 3) and the standard error. Empty symbols represent outlying values.
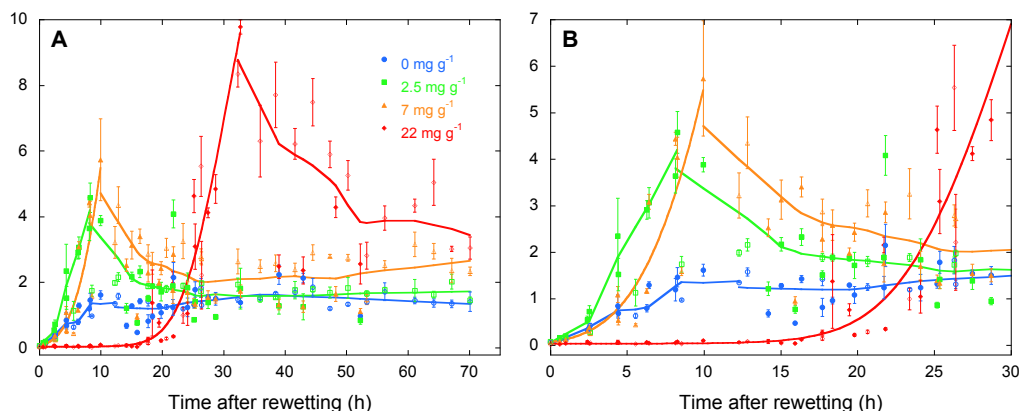
Fig. 4. Bacterial growth, measured as the incorporation of ^{3}H-labelled leucine into bacterial biomass, after rewetting in soils receiving 0, 2.5, 7.3 and 22 mg NaCl g^{-1}. Bacterial growth rates have been normalized to the mean of the rates measured in the continuously moist control (CMC) at each salinity level. Panel A shows the entire length of the experiment (70 h), panel B zooms in on the first 30 h after rewetting. The initial increase in growth was described with a Gompertz model in the 7.3 and 22 mg NaCl g^{-1} treatments ($R^2 = 0.81$ and 0.91), while a smoothing function using the locally weighted least squared error (Lowess) with a smoothing factor of 50% was used to describe growth in the 0 and 2 mg NaCl g^{-1} treatments, as well as in the 7 and 22 mg NaCl g^{-1} treatments after the growth peak. The decline in growth in the 2.5–22 mg NaCl g^{-1} treatments after the peak in growth rate was described by an exponential decay function (R^2 between 0.46 and 0.51). The graphs include measurements from soil sampled in May (open symbols) and June (closed symbols). Data points show the mean (n = 3) and the standard error of the mean.

respiration start to increase until around 20 h after rewetting. The difference between respiration rates in the drying-rewetting and continuously moist control soils increased with salinity in the 0, 2 and 7 mg NaCl g^{-1} treatments, with highest respiration rates being ca. 4, 6 and 10 times higher than the rate in the continuously moist control (Figs. 7 and 8b). In the 22 mg NaCl g^{-1} drying-rewetting treatment respiration rate compared to the continuously moist control was not increased further, however. While in the 0–7 mg NaCl g^{-1} treatments respiration rates were higher than in the continuously moist control throughout the whole experiment (until 70 h after rewetting), in the 22 mg NaCl g^{-1} treatment respiration rates were initially lower than in the continuously moist control and only surpassed the respiration in the continuously moist control ca. 5 h after rewetting (Figs. 7 and 8c).

Similar to bacterial growth, cumulative respiration in the continuously moist control also decreased with increasing concentration of added salt (Fig. 9a). In samples from both May and June respiration in drying-rewetting treatments increased compared to the continuously moist control (Fig. 9b). The difference between the cumulative respiration in drying-rewetting treatments and continuously moist control treatments was higher in more saline soils.

4. Discussion

4.1. Salt influence on microbial biomass and PLFA composition

Salt additions did not have a significant effect on the amount of microbial biomass detected in the different treatments (Table 1). However, the PLFA composition differed markedly between salt treatments (Fig. 2a and b), indicating that a shift in community composition was induced by salt addition, while biomass remained at a relatively constant level. The ratio of fungal to bacterial PLFAs was unaffected by salinity (Table 1). Some bacterial PLFAs, namely i15:0, cy17:0, cy19:0 and 18:1ω7, decreased with higher salinity, whereas other bacterial PLFAs, including a17:0 and 17:0, increased with salinity (Fig. 2b), suggesting that bacterial groups varied in their sensitivity to salt addition.

4.2. Salt influence on the moisture dependence of bacterial growth

We hypothesized (H1) that high salt concentrations would increase the severity of drying experienced by the microbial community. In line with this hypothesis, higher salinity exacerbated the inhibitory effect of drying on bacterial growth (Fig. 3b). At a given level of drought exposure, the inhibition of bacterial growth increased with higher salinity. This indicated that the additional effect of high salt concentrations in the saline treatments during drying exerted a stronger effect on soil bacteria than drying in isolation, resulting in more severe drying affecting the bacterial community. A similar pattern can be seen when modeling bacterial growth against water potential (Fig. S3b). As water potential increases (becomes more negative) during drying, the inhibition of growth is more severe in the 7 and 22 mg NaCl g^{-1} treatments. As such, we met the criteria to test our second hypothesis, and could thus proceed to test how the severity of drying affects drying-rewetting responses.

4.3. Direct effect of salt on the bacterial growth response

We hypothesized (H2) that more severe drying caused by salinity would induce a shift from a type 1 response in less saline soils to a type 2 response of bacterial growth to drying-rewetting in the more saline soils (Fig. 1). We observed a typical type 1 response in the 0 and 2 mg NaCl g^{-1} treatments with a linear increase in bacterial growth starting immediately after rewetting. In the treatments with 7 and 22 mg NaCl g^{-1} we observed a lag period of no growth, after which bacterial growth rates increased exponentially (Fig. 4a and b), similar to type 2 responses previously observed by Göransson et al. (2013) and Meisner et al. (2013, 2015). The duration of this lag period increased with higher salt concentration in the soil (Fig. 5a). These findings supported our hypothesis. While there were differences in cumulative growth between drying-rewetting samples from May and samples from June, with cumulative growth higher in May than in June, the response patterns to salinity were consistent in samples from both time points, adding the power of repeated experiments to the study.
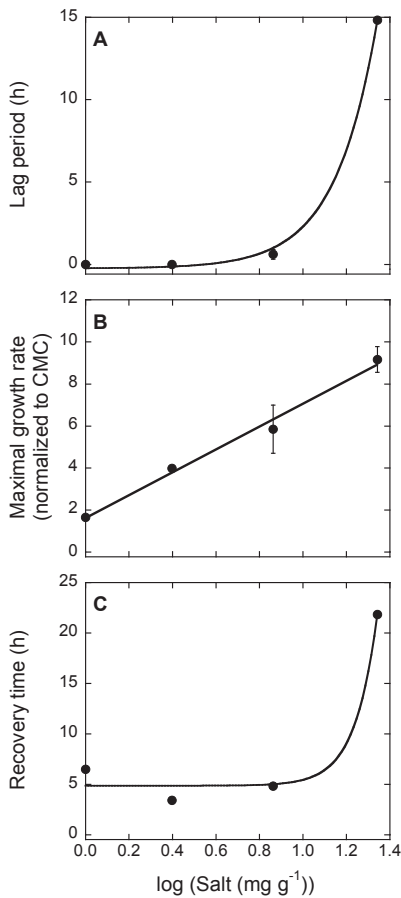
Fig. 5. Panel A shows the lag period for bacterial growth after rewetting. When there was no detectable lag period, duration of the lag period was set to 0. Panel B depicts the maximal bacterial growth rate reached after rewetting. Growth has been normalized to the rates measured in the moist control (CMC) at each salinity level. Panel C shows the recovery time for bacterial growth, which is the duration until rates reached those in the CMC after rewetting. Error bars indicate the standard error of the mean (n = 3).

A type 1 response to drying-rewetting for bacterial growth was observed by Iovieno and Bååth (2008) and Meisner et al. (2013, 2015) in soils dried for short periods of time before rewetting and has been interpreted as indicative of drying that was experienced as relatively mild by the microbial community (Meisner et al., 2015). The lag period of bacterial growth that characterizes a type 2 response to drying-rewetting (Fig. 1) was previously observed by Göransson et al. (2013) and Meisner et al. (2013, 2015). In Meisner et al. (2013, 2015) the severity of drying was increased by extending the duration of drought before rewetting, while in the case of our experiments severity of drying experienced by the microbial community was increased by high concentrations of dissolved salts in the soil solution that accumulated during drying. As previously discussed (Meisner et al., 2013, 2015), the lag period could be explained by a low number of active microorganisms remaining at the end of a more severe drying period. In bacterial cultures, growth generally exhibits an initial lag phase before growth accelerates
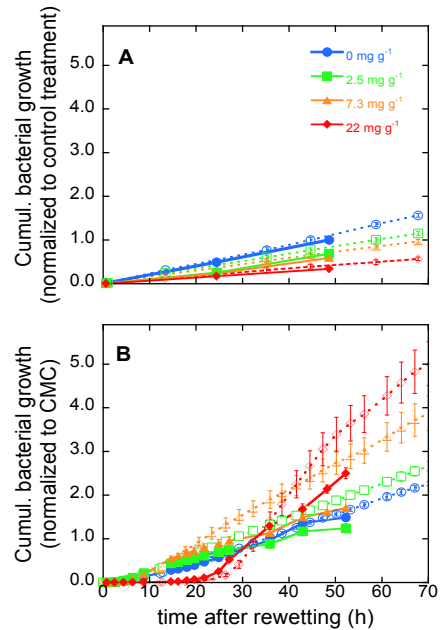

Fig. 6. Panel A shows cumulative bacterial growth in the continuously moist control (CMC). Growth has been normalized to the value in the control treatment without salt at 50 h. Panel B shows cumulative bacterial growth in drying-rewetting treatments of different salinities (0, 2.5, 7 and 22 mg NaCl g^{-1}), normalized to the cumulative growth in the CMC at 50 h (Panel A) at each salinity level. Open symbols represent the set of microcosms set up with soil sample from May 2015, closed symbols the second set of microcosms containing soil sample from June 2015. Error bars indicate the standard error of the mean (n = 3).
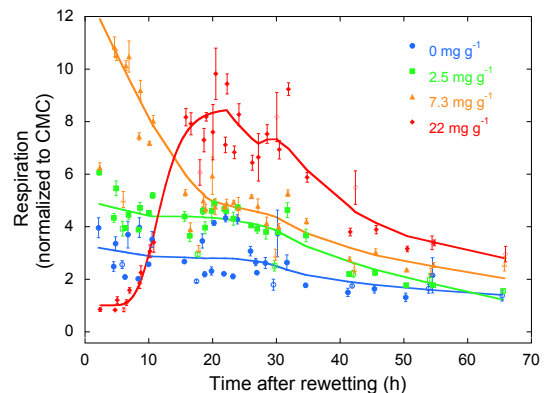

Fig. 7. Respiration after rewetting in soils receiving 0, 2.5, 7.3 and 22 mg NaCl g^{-1}. Respiration rates have been normalized to the rates measured in the continuously moist control (CMC) at each salinity level. Initial respiration until the maximal respiration in the 22 mg NaCl g^{-1} treatment was modeled with a Gompertz function ($R^2 = 0.98$), while a smoothing function using the locally weighted least squared error (Lowess) with a smoothing factor of 50% was used to describe respiration in the 0–7 mg NaCl g^{-1} treatments, as well as in the 22 mg NaCl g^{-1} treatments after the respiration peak. The figure includes measurements from soil sampled in May (open symbols) and June (closed symbols). Data points show the mean (n = 3) and the standard error.
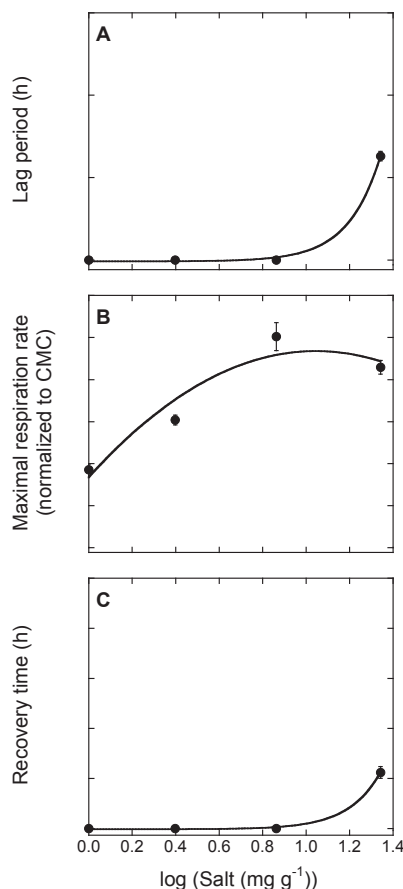
Fig. 8. Panel A shows the lag period for respiration after rewetting. When there was no detectable lag period, duration of the lag period was set to 0. Panel B depicts the maximal respiration rate reached after rewetting. Respiration has been normalized to the rates measured in the moist control (CMC) at each salinity level. Panel C shows the recovery time for respiration, which is the duration until rates reached those in the CMC after rewetting. Recovery time is set to 0 in cases where respiration rates surpassed respiration in the CMC immediately after rewetting. Error bars indicate the standard error of the mean (n = 3).
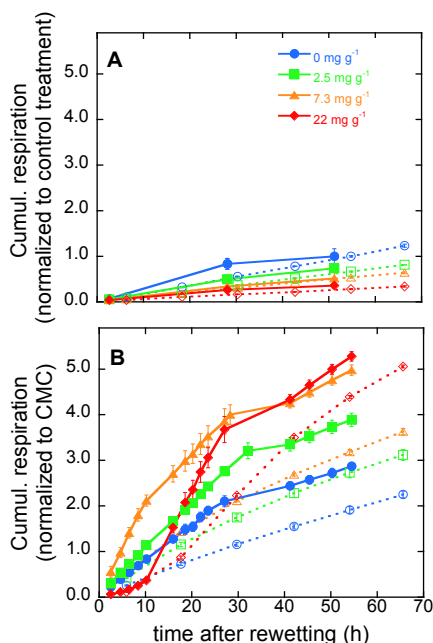

Fig. 9. Panel A shows cumulative respiration in the continuously moist control (CMC). Respiration has been normalized to the value in the control treatment without salt at 50 h. Panel B shows cumulative respiration in drying-rewetting treatments of different salinities (0, 2.5, 7 and 22 mg NaCl g^{-1}), normalized to the cumulative respiration in the CMC at 50 h (Panel A) at each salinity level. Empty diamonds represent the set of microcosms set up with soil sample from May 2015, full circles the second set of microcosms containing soil sample from June 2015. Error bars indicate the standard error of the mean (n = 3).

exponentially (Monod, 1949; Zwietering et al., 1990). This lag phase has been shown to increase after cells have been damaged and in response to reduced inoculum size (Mackey and Derrick, 1982; Pascual et al., 2001). If the mechanism triggering the switch from a type 1 to a type 2 response to drying-rewetting is based on the size and the physiological status of the community that survives the drying event (Meisner et al., 2013, 2015), then independent ways of suppressing the microbial community should all result in consistent increases in the lag period. Our results are in accordance with this expectation. As such, the reduction in the number of surviving and active microorganisms during drying leading to the lag period in growth is a consequence of salt during drying.

4.4. Salt effect on the respiration pulse following drying-rewetting

The connection between the respiratory response to drying-

rewetting and bacterial growth observed here is different from previous work (Göransson et al., 2013; Meisner et al., 2013, 2015). While in the 0–7 mg NaCl g^{-1} treatments drying-rewetting led to a pronounced respiration pulse immediately following rewetting, the respiration rates in the 22 mg NaCl g^{-1} drying-rewetting treatment were initially suppressed below the level in the continuously moist control treatment and onset of a respiration pulse occurred only after a lag period (Figs. 7 and 8a). This type 2 respiration response has never previously been observed. In a typical type 2 respiratory response (Meisner et al., 2013, 2015) rates after drying-rewetting are higher than in the continuously moist control, sustained at those high levels, and then sometimes increase further at the end of the lag period when growth begins to increase (Fig. 1). While reductions in the amplitude of the respiration pulse after drying-rewetting in saline soils compared to non-saline soils have been found in other studies (Chowdhury et al., 2011b; Mavi and Marschner, 2012), no lag period in the onset of the respiration pulse has been reported before, possibly due to a lack of temporal resolution. The peak respiration rates in the 22 mg NaCl g^{-1} treatment occurred 15–20 h after rewetting (Fig. 7), which coincided with the end of the lag period of bacterial growth.

Many mechanisms, both biological processes mediated by the metabolic activity of the soil community and physical processes such as the displacement of gases from soil pores have been put forward to explain the respiration pulse observed after drying-rewetting (Kim et al., 2012). One biological process that has been proposed as a possible mechanism for the respiration pulse

observed after rewetting is extracellular activity of enzymes (Miller et al., 2005; Lawrence et al., 2009; Evans et al., 2016). Extracellular enzymes can remain active during the drying period and gain access to available substrate following rewetting. Their activity during drying and immediately after rewetting may provide an enlarged pool of labile organic matter to surviving cells (Lawrence et al., 2009; Moyano et al., 2013). In addition, intracellular enzymes are also released into the soil solution from lysed cells. It has been suggested that these respiratory enzymes can persist in soil and even maintain an extracellular oxidative metabolism under conditions without microbial life (Maire et al., 2013; Fraser et al., 2016). Once a dried soil is rewetted, these enzymes could gain rapid access to newly available resources, leading to a pulse in respiration.

Salt reduced microbial activity and resource use in non-tolerant communities (also see next section below) and therefore more plant litter resource still remained unused in the most saline soils. We expected this to lead to higher resource availability to microorganisms once the microbial community had adapted to the saline conditions and therefore a higher resource availability after rewetting in the most saline treatment could fuel a higher respiration pulse. Indeed, in the 0–7 mg NaCl g^{-1} treatments the respiration pulse relative to the continuously moist control increased with salinity (Figs. 7 and 8b). However, in the most saline treatment microorganisms seemed to have been unable to immediately use the available substrate following rewetting (Fig. 7), which could be explained by an inhibition of enzymatic activity in saline soils during drying (see discussion above). It is likely that the high salt concentrations that developed in the soil solution of the highly saline treatment during drying (>6 M NaCl) could have led to denaturation and "salting out" of proteins and inhibited the activity of these enzymes (Jaenicke, 1991; Zidani et al., 2012). Thus, upon rewetting the activity of "resident" enzymes able to use the newly available substrate could have been reduced in the highly saline treatment, leading to a lower respiration rate. If enzymatic inhibition led to the reduction in the respiration pulse upon rewetting, respiration should only increase after new enzymes could be synthesized to replace the denatured stock, which would have coincided with the recovery of bacterial growth (Fig. 4). Our results are compatible with this explanation, suggesting a link between respiration increase and bacterial growth in the 22 mg NaCl g^{-1} treatment.

The cumulative respiration rate in the drying-rewetting treatment was higher in the sample collected in June than in May (Fig. 9b). This difference could not be explained by any of the measured soil properties, including OM content, pH and $EC_{1:5}$, which did not differ between soils from both sampling time points. However, the activity and physiological state of the microbial community could have been affected by one or several unmeasured factors such as plant productivity or phenology, leading to a larger increase in respiration following drying-rewetting in the sample collected in June. Despite the differences in cumulative respiration between the two sampling time points, a similar pattern emerged with soils that received a greater addition of NaCl having a more strongly increased cumulative respiration after drying-rewetting compared to the continuously moist control than less saline soils (Fig. 9b). This suggests that factors that influenced the amplitude of the response to drying-rewetting did not have an impact on the direction of the response with increasing salinity.

4.5. Indirect effect of salt through altered resource availability

In addition to the direct effect of salt on respiration and growth, salt could also indirectly influence the drying-rewetting response of soils. The increased respiration and growth in drying-rewetting treatments compared to the continuously moist control in saline

soils (Fig. 9) as well as the exponential increase in bacterial growth following the lag period (Fig. 4) could have been fuelled by a higher availability of resources (also see discussion above). Several processes could have contributed to higher resource availability in the highly saline soils. Firstly, since microbial processes had been partially inhibited in the more saline treatments over the course of the incubation period, it is likely that at the time of the drying-rewetting experiment more of the plant material added at the beginning of the incubation period remained in the soil. In addition to the higher amount of remaining plant material, higher ion concentrations in the soil solution increased the extraction of dissolved organic carbon (DOC) from plant material and soil (Jones and Willett, 2006). Thus, saline soils can contain higher concentrations of DOC than non-saline soils following drying-rewetting (Mavi and Marschner, 2012), which could suggest a higher availability of labile C. Secondly, the more saline soils could also have contained higher concentrations of labile compounds derived from killed microbial cells. In order to cope with high salt concentrations in the soil, microorganisms are thought to synthesize and accumulate osmolytes (Schimel et al., 1989; Kakumanu and Williams, 2014). Through death and lysis of cells during drying-rewetting, these osmolytes, together with other low molecular weight compounds, are released into the soil environment and become available as substrate. Diffusion limitation during drying could limit the access of microorganisms to the resources needed to synthesize osmolytes (Manzoni et al., 2016), resulting in a lower osmolyte production in response to low soil water content compared to high salt concentrations (Kakumanu and Williams, 2014). With a higher amount of substrate remaining in the more saline treatment, it is possible that the microorganisms had better access to the resources required to synthesize osmolytes, as well as a greater need for osmolytes in order to resist salinity. Higher pre-drought osmolyte accumulation combined with increased cell death in saline soils undergoing drying-rewetting could lead to more cytoplasmatic compounds being released into the soil environment and thus contribute to an increased availability of DOC in saline soils. As such, it could be speculated that osmolyte turnover could explain a larger fraction of the respiration pulse in saline soils after drying-rewetting. Following from this line of reasoning, drying-rewetting events may have a higher potential to increase respiration fluxes in saline soils than in soils with low salinity, suggesting a higher potential for losses of C during drying-rewetting cycles in saline soils. It needs to be noted that soils in this study were supplied with additional plant substrate, whereas saline soils often contain lower quantity and quality of organic matter than non-saline soils (Setia et al., 2013; Rath and Rousk, 2015) introducing important caveats to consider. In addition to its impact on C dynamics during drying-rewetting, lower resource availability could also affect the ability of microbial communities to adapt to short-term changes of environmental conditions (Hasbullah and Marschner, 2015), thereby modifying the microbial response to drying-rewetting events.

5. Conclusion

We found that high soil salinity increased the level of inhibition by low moisture, providing evidence that the severity of drying experienced by the microbial community in saline soil increased compared to drying in non-saline soil. The severity of drying induced by salinity could also cause a change from a type 1 to a type 2 response of bacterial growth to rewetting (Fig. 1). This change was consistent with results from previous studies where severity of drying was increased by other means. We also found an interaction where the effect of drying-rewetting on bacterial growth and respiration increased with higher salinity. At very high salinity we observed that a lag period for respiration coincided with a lag

period for growth. This created a transient link between the rate of respiration and bacterial growth during the lag period and subsequent exponential increase in growth never reported previously. We speculate that salt-induced precipitation of resident exoenzymes and subsequent replacement with newly synthesized protein caused this link.

Acknowledgements

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2017.01.018.

References

Bååth, E., 1994. Thymidine and leucine incorporation in soil bacteria with different cell size. Microbial Ecology 27, 267—278.

Bååth, E., Pettersson, M., Söderberg, K.H., 2001. Adaptation of a rapid and economical microcentrifugation method to measure thymidine and leucine incorporation by soil bacteria. Soil Biology and Biochemistry 33, 1571—1574.

Brady, N.C., Weil, R.R., 2002. The Nature and Properties of Soils, thirteenth ed. Prentice Hall, Upper Saddle River, N.J.

Chowdhury, N., Nakatani, A.S., Setia, R., Marschner, P., 2011a. Microbial activity and community composition in saline and non-saline soils exposed to multiple drying and rewetting events. Plant and Soil 348, 103—113.

Chowdhury, N., Yan, N., Islam, M.N., Marschner, P., 2011b. The extent of drying influences the flush of respiration after rewetting in non-saline and saline soils. Soil Biology & Biochemistry 43, 2265—2272.

Evans, S., Dieckmann, U., Franklin, O., Kaiser, C., 2016. Synergistic effects of diffusion and microbial physiology reproduce the Birch effect in a micro-scale model. Soil Biology & Biochemistry 93, 28—37.

Fraser, F.C., Corstanje, R., Deeks, L.K., Harris, J.A., Pawlett, M., Todman, L.C., Whitmore, A.P., Ritz, K., 2016. On the origin of carbon dioxide released from rewetted soils. Soil Biology & Biochemistry 101, 1—5.

Frostegård, A., Bååth, E., 1996. The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. Biology and Fertility of Soils 22, 59—65.

Frostegård, A., Bååth, E., Tunlid, A., 1993. Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty-acid analysis. Soil Biology & Biochemistry 25, 723—730.

Gibson, A.M., Bratchell, N., Roberts, T.A., 1988. Predicting microbial growth: growth responses of salmonellae in a laboratory medium as affected by pH, sodium chloride and storage temperature. International Journal of Food Microbiology 6, 155—178.

Göransson, H., Godbold, D.L., Jones, D.L., Rousk, J., 2013. Bacterial growth and respiration responses upon rewetting dry forest soils: impact of drought-legacy. Soil Biology and Biochemistry 57, 477—486.

Hasbullah, H., Marschner, P., 2015. Residue properties influence the impact of salinity on soil respiration. Biology and Fertility of Soils 51, 99—111.

Iovieno, P., Bååth, E., 2008. Effect of drying and rewetting on bacterial growth rates in soil. Fems Microbiology Ecology 65, 400—407.

IUSS Working Group WRB, 2006. World Reference Base for Soil Resources, 2006 ed. FAO, Rome.

Jaenicke, R., 1991. Protein stability and molecular adaptation to extreme conditions. European Journal of Biochemistry 202, 715—728.

Jones, D.L., Willett, V.B., 2006. Experimental evaluation of methods to quantify dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) in soil. Soil Biology & Biochemistry 38, 991—999.

Kakumanu, M.L., Williams, M.A., 2014. Osmolyte dynamics and microbial communities vary in response to osmotic more than matric water deficit gradients in two soils. Soil Biology and Biochemistry 79, 14—24.

Kim, D.G., Vargas, R., Bond-Lamberty, B., Turetsky, M.R., 2012. Effects of soil rewetting and thawing on soil gas fluxes: a review of current literature and suggestions for future research. Biogeosciences 9, 2459—2483.

Lawrence, C.R., Neff, J.C., Schimel, J.P., 2009. Does adding microbial mechanisms of decomposition improve soil organic matter models? A comparison of four models using data from a pulsed rewetting experiment. Soil Biology & Biochemistry 41, 1923—1934.

Mackey, B.M., Derrick, C.M., 1982. The effect of sublethal injury by heating, freezing, drying and gamma-radiation on the duration of the lag phase of Salmonella-Typhimurium. Journal of Applied Bacteriology 53, 243—251.

Maheshwari, A., 2015. Assessing Soil Microbial Tolerance to Salt. Department of Biology. Lund University, Lund, Sweden.

Maire, V., Alvarez, G., Colombet, J., Comby, A., Despinasse, R., Dubreucq, E., Joly, M., Lehours, A.C., Perrier, V., Shahzad, T., Fontaine, S., 2013. An unknown oxidative metabolism substantially contributes to soil CO2 emissions. Biogeosciences 10, 1155—1167.

Manzoni, S., Moyano, F., Kätterer, T., Schimel, J., 2016. Modeling coupled enzymatic and solute transport controls on decomposition in drying soils. Soil Biology and Biochemistry 95, 275—287.

Mavi, M.S., Marschner, P., 2012. Drying and wetting in saline and saline-sodic soils-effects on microbial activity, biomass and dissolved organic carbon. Plant and Soil 355, 51—62.

Meisner, A., Bååth, E., Rousk, J., 2013. Microbial growth responses upon rewetting soil dried for four days or one year. Soil Biology & Biochemistry 66, 188—192.

Meisner, A., Rousk, J., Bååth, E., 2015. Prolonged drought changes the bacterial growth response to rewetting. Soil Biology & Biochemistry 88, 314—322.

Miller, A.E., Schimel, J.P., Meixner, T., Sickman, J.O., Melack, J.M., 2005. Episodic rewetting enhances carbon and nitrogen release from chaparral soils. Soil Biology & Biochemistry 37, 2195—2204.

Monod, J., 1949. The growth of bacterial cultures. Annual Review of Microbiology 3, 371—394.

Moyano, F.E., Manzoni, S., Chenu, C., 2013. Responses of soil heterotrophic respiration to moisture availability: an exploration of processes and models. Soil Biology & Biochemistry 59, 72—85.

Pascual, C., Robinson, T.P., Ocio, M.J., Aboaba, O.O., Mackey, B.M., 2001. The effect of inoculum size and sublethal injury on the ability of Listeria monocytogenes to initiate growth under suboptimal conditions. Letters in Applied Microbiology 33, 357—361.

Rath, K.M., Rousk, J., 2015. Salt effects on the soil microbial decomposer community and their role in organic carbon cycling: a review. Soil Biology & Biochemistry 81, 108—123.

Rengasamy, P., 2006. Soil salinity and sodicity. In: Stevens, D. (Ed.), Growing Crops with Reclaimed Wastewater. CSIRO Publishing, Collingwood, Australia, pp. 125—138.

Richards, L.A., 1954. Diagnosis and improvement of saline and alkali soils. Soil Science 78, 154.

Rousk, J., Bååth, E., 2007. Fungal and bacterial growth in soil with plant materials of different C/N ratios. Fems Microbiology Ecology 62, 258—267.

Schimel, J.P., Scott, W.J., Killham, K., 1989. Changes in cytoplasmic carbon and nitrogen pools in a soil bacterium and a fungus in response to salt stress. Applied and Environmental Microbiology 55, 1635—1637.

Setia, R., Gottschalk, P., Smith, P., Marschner, P., Baldock, J., Setia, D., Smith, J., 2013. Soil salinity decreases global soil organic carbon stocks. Science of The Total Environment 465, 267—272.

Soil Survey Staff, 1999. Soil Taxonomy: a Basic System of Soil Classification for Making and Interpreting Soil Surveys. In: Natural Resources Conservation Service, second ed. U.S. Department of Agriculture., Washington, DC.

Zidani, S., Fahloul, D., Bacha, A., 2012. Effects of pH, NaCl, ethanol, and drying methods on the solubility of Saccharomyces cerevisiae proteins. CyTA - Journal of Food 10, 42—47.

Zwietering, M.H., Jongenburger, I., Rombouts, F.M., Vantriet, K., 1990. Modeling of the bacterial growth curve. Applied and Environmental Microbiology 56, 1875—1881.

Supplemental information for Paper VI

Table S1: Respiration and bacterial growth in moist soil of different salinities at the onset of the drying experiment. Numbers in parentheses are 1 standard error of the mean (n=3).

	NaCl			
	0 mg g^{-1}	2.5 mg g^{-1}	7.3 mg g^{-1}	22 mg g^{-1}
Respiration (μg CO_2 g^{-1} h^{-1})	1.22 (0.05)	1.10 (0.07)	0.99 (0.05)	0.50 (0.04)
Bacterial growth (pmol Leu g^{-1} h^{-1})[1]	54.3 (2.3)	53.6 (5.6)	74.5 (16.4)	177.0 (18.8)

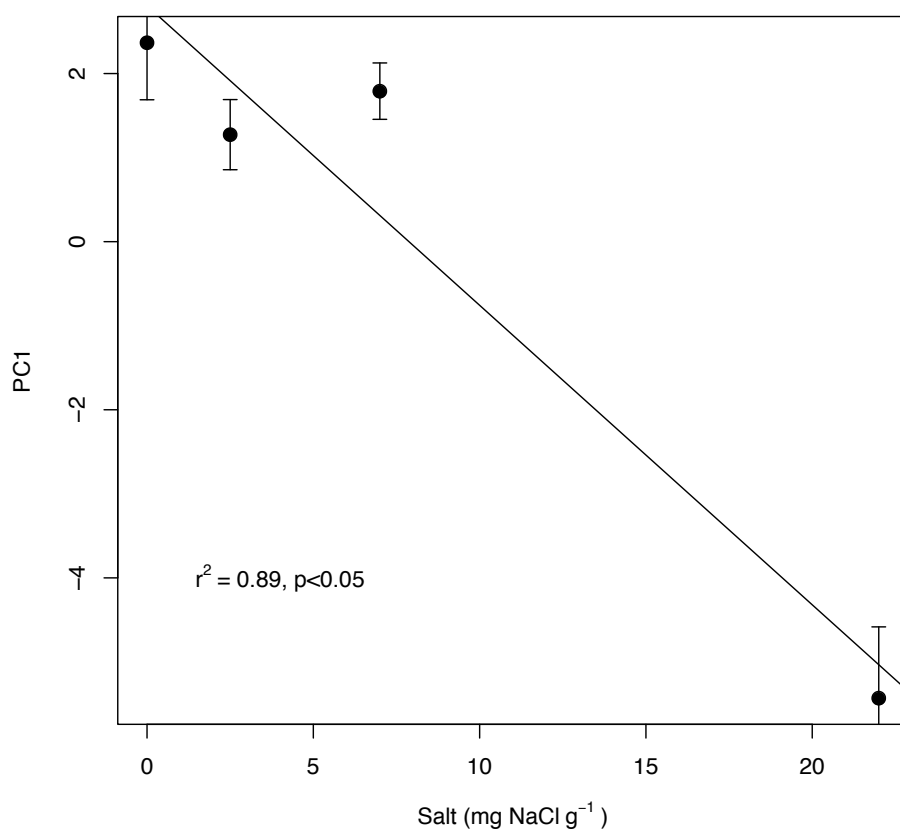[1] measured as the incorporation of ^{3}H-labelled leucine into bacterial biomass

Fig. S1: Linear regression between salt addition and principal component 1 (PC1) in a principal component analysis (PCA) performed on the phospholipid fatty acid (PLFA) composition of samples.
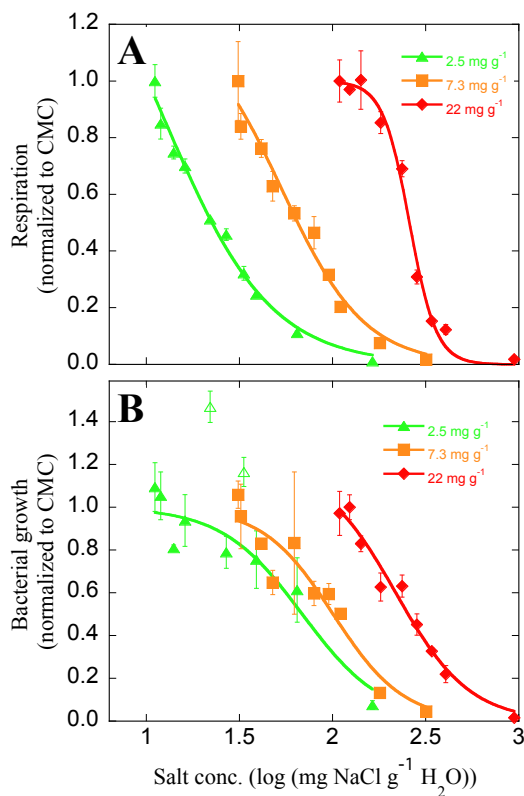
Fig. S2: Logistic inhibition curves of respiration (A), and bacterial growth (B) during drying of soil against accumulating NaCl concentrations in the remaining soil water (r^2 between 0.91 and 0.99). Relative process rates have been normalized to measurements in moist samples. Salt concentrations are given as the common logarithm of the concentration. Data points represent the mean and the standard error of the mean (n=3). The salt concentration during drying in the 0 mg NaCl g^{-1} treatment could not be determined and the treatment was excluded from this graph.
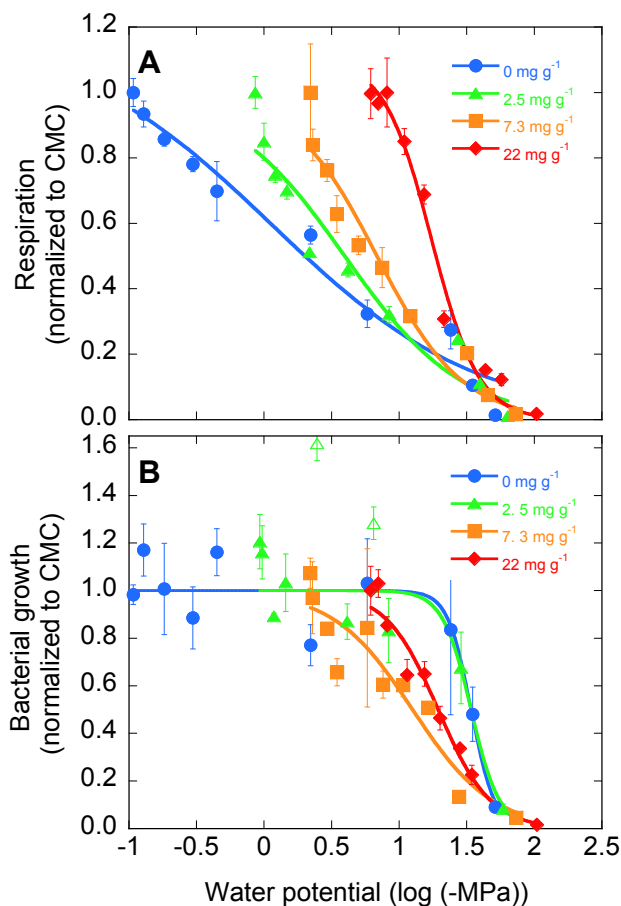
162

Fig. S3: Logistic inhibition curves of respiration (A), and bacterial growth (B) during drying of soil against total water potential (r^2 between 0.86 and 0.98). Relative process rates have been normalized to measurements in moist samples. Water potentials have been multiplied by -1 and are given as the common logarithm. Data points represent the mean and the standard error of the mean (n=3).

DOCTORAL THESES PUBLISHED IN ENVIRONMENTAL SCIENCE, LUND UNIVERSITY

1. Georg K.S. Andersson (2012) Effects of farming practice on pollination across space and time. Department of Biology/Centre for environmental and climate research

2. Anja M. Ödman (2012) Disturbance regimes in dry sandy grasslands – past, present and future. Department of Biology/ Centre for environmental and climate research

3. Johan Genberg (2013) Source apportionment of carbonaceous aerosol. Department of Physics/ Centre for environmental and climate research

4. Petra Bragée (2013) A palaeolimnological study of the anthropogenic impact on dissolved organic carbon in South Swedish lakes. Department of Geology/ Centre for environmental and climate research

5. Estelle Larsson (2013) Sorption and transformation of anti-inflammatory drugs during wastewater treatment. Department of Chemistry/ Centre for environmental and climate research

6. Magnus Ellström (2014) Effects of nitrogen deposition on the growth, metabolism and activity of ectomycorrhizal fungi. Department of Biology/ Centre for environmental and climate research

7. Therese Irminger Street (2015) Small biotopes in agricultural landscapes: importance for vascular plants and effects on management. Department of physical geography and ecosystem science/ Department of Biology/ Centre for environmental and climate research

8. Helena I. Hanson (2015) Natural enemies: Functional aspects of local management in agricultural landscapes. Department of Biology/ Centre for environmental and climate research

9. Lina Nikoleris (2016) The estrogen receptor in fish and effects of estrogenic substances in the environment: ecological and evolutionary perspectives and societal awareness Department of Biology/ Centre for environmental and climate research

10. Cecilia Hultin (2016) Estrogen receptor and multixenobiotic resistance genes in freshwater fish and snails: identification and expression analysis after pharmaceutical exposure. Centre for environmental and climate research

11. Annika M. E. Söderman (2016) Small biotopes: Landscape and management effects on pollinators. Department of Biology/ Centre for environmental and climate research

12. Wenxin Ning (2016) Tracking environmental changes of the Baltic Sea coastal zone since the mid-Holocene. Department of Geology/ Centre for environmental and climate research

13. Karin Mattsson (2016) Nanoparticles in the aquatic environment, Particle characterization and effects on organisms. Department of Chemistry/ Centre for environmental and climate research

14. Ola Svahn (2016) Tillämpad miljöanalytisk kemi för monitorering och åtgärder av antibiotika- och läkemedelsrester I Vattenriket. School of Education and Environment, Kristianstad University/ Centre for environmental and climate research

15. Pablo Urrutia Cordero (2016) Putting food web theory into action: Local adaptation of freshwaters to global environmental change. Department of Biology/ Centre for environmental and climate research

16. Lin Yu (2016) Dynamic modelling of the forest ecosystem: Incorporation of the phosphorous cycle. Centre for environmental and climate research

17. Behnaz Pirzamanbein (2016) Recontruction of past European land cover based on fossil pollen data: Gaussian Markov random field models for compositional data. Centre for Mathematical Sciences/ Centre for environmental and climate research

18. Arvid Bolin (2017) Ecological interactions in human modified landscapes – Landscape dependent remedies for the maintenance of biodiversity and ecosystem services. Department of Biology/ Centre for environmental and climate research

19. Johan Martinsson (2017) Development and Evaluation of Methods in Source Apportionment of the Carbonaceous Aerosol. Department of Physics/ Centre for environmental and climate research

20. Emilie Öström (2017) Modelling of new particle formation and growth in the atmospheric boundary layer. Department of Physics/ Centre for environmental and climate research

21. Lina Herbertsson (2017) Pollinators and Insect Pollination in Changing Agricultural Landscapes. Centre for environmental and climate research

22. Sofia Hydbom (2017) Tillage practices and their impact on soil organic carbon and the microbial community. Department of Biology/ Centre for environmental and climate research

23. Erik Ahlberg (2017). Speeding up the Atmosphere: Experimental oxidation studies of ambient and laboratory aerosols using a flow reactor. Department of Physics/Centre for environmental and climate research

24. Laurie M. Charrieau (2017) DISCO - Drivers and Impacts of Coastal Ocean Acidification. Department of Geology/ Centre for Environmental and Climate Research

25. Kristin Rath (2018). Soil salinity as a driver of microbial community structure and functioning. Department of Biology/ Centre for Environmental and Climate Research