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# Impact of salinity on soil microbial communities and the decomposition of maize in acidic soils

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**Abstract**

Soil salinity, as an increasingly important process of land degradation, is a major threat to microbial communities and thus strongly alters organic matter turnover processes. This study was conducted to determine the influence of salinity on the decomposition of maize and on the response of soil microbial communities. Soil samples were collected from two pasture sites in Heringen (Germany). One of the sites has previously been influenced by salinity caused by saline effluent from a potassium mine. These sandy soils were washed, resulting in equal levels of electrical conductivity. Moist soils were then incubated with 2% incorporated maize straw and at three levels of salinity (0, 15, 50 mg NaCl $g^{-1}$ soil) for almost 7 weeks at 25 °C. The amount of recovered maize derived particulate organic matter (POM) increased with increasing salinity, exhibiting reduced decomposition of substrate. Furthermore, inorganic N, which consisted almost exclusively of $NH_4^+$, increased with increasing levels of salinity. Corresponding to this, biological indices like soil respiration and microbial biomass decreased with increasing levels of salinity, underlining the detrimental effect of salinity on soil microorganisms. This effect was reduced after addition of maize straw, documenting the importance of organic matter amendment in counteracting the negative effects of salinity on microbial communities and related mineralisation processes. Addition of organic matter also led to a spatial differentiation of the microbial community in the soil, with bacteria dominating the surface of the substrate, indicated by a low glucosamine-to-muramic acid ratio. This ratio, however, was not altered by salinity. On the other hand, the ergosterol-to-microbial biomass C ratio was an evidence of fungal dominance in the soil. The ratio increased with elevated salt content, either showing a shift towards fungi, a change in fungal cell morphology, or accumulation of ergosterol in the soil. The metabolic quotient $qCO_2$ was higher in the soil previously subjected to osmotic stress, showing a physiologically more active population that is using substrate less efficiently. We assume that it might further reflect adaptation mechanisms to the increased osmotic pressure.


*Keywords:* Amino sugars; Fungi-to-bacteria ratio; Microbial activity; Osmotic stress; Physiological adaptation; Salinisation

## 1. Introduction

Soil salinity is part of natural ecosystems under arid and semi-arid conditions (Pathak and Rao, 1998), and an increasing problem in agricultural soils throughout the world (Keren, 2000; Qadir et al., 2000). In temperate humid climates soil salinity occurs on a smaller scale, mainly in salt marshes, along roads, and on saline waste dumps.

The influence of salt as a major stress to soil microorganisms, which is more potent than that of heavy metals (Sardinha et al., 2003), has been the subject of several studies (e.g. Sarig and Steinberger, 1994; Pankhurst et al., 2001; Mamilov et al., 2004). A decrease in $CO_2$-evolution, enzymatic activity, or microbial biomass has often been observed (e.g. Laura, 1974; Pathak and Rao, 1998; Rietz and Haynes, 2003). Increasing salinity thus has detrimental effects on biologically mediated processes in the soil, such as C and N-mineralisation (Pathak and Rao, 1998). In saline soils and under drought, microbes suffer from osmotic stress, which results in drying and lysis of cells.

Despite this, soil microorganisms have the ability to adapt to or tolerate osmotic stress caused by drought or salinity, especially when regularly confronted with such conditions (Sparling et al., 1989). There are also examples of microbes thriving in strongly saline ponds (e.g. Casamayor et al., 2002), documenting the evolutionary potential of microorganisms. However, fungi tend to be sensitive to salt stress, indicated by

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decreasing ergosterol contents in the soil (Sardinha et al., 2003). Van Bruggen and Semenov (2000) reported that long-term stress results in decreasing fungal diversity. On the other hand, Killham (1994) mentioned that filamentous fungi are highly tolerant to water stress. They nevertheless have to cope with increasing osmotic pressure and thus might change their physiology (Killham, 1994) and morphology in response to this (Zahran, 1997). Killham (1994) describes two main adaptation strategies of microorganisms to osmotic stress, such as salinity, drought or freezing, all of which result in accumulation of solutes in the cell to counteract the increased osmotic pressure. One is to selectively exclude the incorporated solute (e.g. $Na^+$, $Cl^-$) and thus accumulate other ions necessary for metabolism instead (e.g. $NH_4^+$). The other adaptation mechanism of the cell is to produce organic compounds which will antagonise the concentration gradient between soil solution and cell cytoplasm. This adaptation ultimately results in a physiologically more active microbial community and consequently in a lower substrate use efficiency. However, these mechanisms are known from single microorganisms but have hardly ever been studied on a community level. The metabolic quotient as the specific respiration of a resting microbial community (Anderson and Domsch, 1985) provides a suitable tool for investigating the physiological reaction of a microbial community under osmotic stress.

Therefore, we conducted an incubation experiment to study the response of soil microbial communities *in situ* to elevated salinity and the effects on soil microbial properties and related processes. We hypothesised that (1) increasing salinity decreases soil microbial biomass and activity and thus the turnover of organic matter, and that (2) elevated salinity would lead to a stronger decline of fungi than bacteria. We assumed (3) that soil microorganisms in the soil previously prone to salinity are adapted to salt stress, which results in (4) a higher mineralisation of organic matter in comparison to the other soil.

## 2. Materials and methods

### 2.1. Soil sampling and conditioning

Soil samples were taken from two grassland sites with low pH used as meadow. They were situated in the flood plain of the river Werra close to Heringen in North Hessia, Germany, and were approximately 100 m apart. At one site (A) plants showed no symptoms of salinity, whereas the other site (B) has been prone to salinity to a high extent, which was illustrated by no growth of plants and establishment of highly salt tolerant plant species on a neighbouring site (Sardinha et al., 2003). Soil type at site A was classified as a Dystric Fluvisol, whereas soil type at site B was a Salic Fluvisol (Sardinha et al., 2003). Monthly rainfall varies between less than 10 and more than 150 mm, with an average annual rainfall of about 690 mm. For more than 100 years, liquid saline waste from a nearby potassium plant has been injected into underground geological formations that emerged at different sites in this area. The salt was predominantly NaCl (Sardinha et al., 2003). Site A was dominated by *Alopecurus pratensis* L., *Poa pratensis* L., and *Arrhenatherum elatius* L., whereas at site B no vegetation could be detected, but particulate plant residues were present. At a distance of approximately 2 m to site B, small patches of the halophyte *Spegularia salina* L. were observed. At both sites, five core samples (100 $cm^{-3}$) were taken at 0 to 5 cm depth in May 2004 to determine bulk density. In addition, a larger bulk of soil was taken at 0 to 10 cm depth, put into polyethylene bags, transferred to the laboratory in a cooled box immediately, sieved wet (<2 mm) and stored at 4 °C until further processing. Both soils were washed repeatedly to reduce the salt content until the electrical conductivity in the extract was at a low level in the salt affected soil (site B) similar to that in the soil from site A. We assumed that the washing procedure did not influence the microbial biomass, as most soil particles with adhering microorganisms were recovered after washing.

### 2.2. Experimental set-up

An incubation experiment was conducted as a three-factorial design with four replicates of each treatment. The washed soils from sites A and B were weighed (120 g) into 1 litre stoppered Pyrex jars and incubated either with or without 2% (on an oven-dry basis) maize straw (42.9% C, 1% N) addition at three different levels of added salt (0, 15, 50 mg NaCl $g^{-1}$ soil, corresponding to a calculated electrical conductivity of approximately 1, 8 and 24 dS $m^{-1}$). Maize straw was thoroughly mixed into the soil samples. Samples were then incubated for 47 days at 25 °C and approximately 60% water holding capacity in the dark. Soil respiration was measured after 2, 4 and 7 days after incubation commenced and thereafter every 7 days. Then, the evolved $CO_2$ was captured in 5 to 15 ml 1 M NaOH and titrated with 1 M HCl after addition of $BaCl_2$ and phenolphthalein indicator solution. The maize derived $CO_2$–C (μg $d^{-1}$ $g^{-1}$ soil) was estimated by subtracting the soil respiration of the control treatments from the respiration in the respective maize treatments, with the assumption that no priming effects occurred. The specific $CO_2$ evolution of the microbial biomass, the metabolic quotient ($qCO_2$), was calculated from the basal respiration as follows: (μg $CO_2$–C $d^{-1}$ $g^{-1}$ soil evolved during the last 12 days of incubation in the control samples)/(μg microbial biomass C $g^{-1}$ soil at the end of the incubation experiment) × 1000 = mg $CO_2$–C $g^{-1}$ microbial biomass C $d^{-1}$. At the end of the experiment, sub-samples of each treatment were analysed for soil organic C and total N, microbial biomass C and N, ergosterol content and inorganic N.

### 2.3. Measurement of soil chemical and physical properties

Clay content was determined by a pipette method after pretreatment with $H_2O_2$ to remove organic matter (Gee and Bauder, 1986). The pH of the soil was determined before the incubation experiment in water and in 0.01 M $CaCl_2$ using a soil-to-solution ratio of 1:2.5. The cation exchange capacity of the original soils was measured according to Ryan et al. (1996) in sodium acetate solution and in unbuffered 0.1 M $BaCl_2$ solution (Schlichting et al., 1995) as were the washed soils. Exchangeable cations were measured by atomic absorption spectrometry (M series,

Unicam, Kassel). Extractable sodium in the saline soil (soil B) was measured after extraction with calcium acetate lactate by atomic absorption spectrometry. Electrical conductivity ($EC_e$) was measured in the extract of a water saturated paste according to Ryan et al. (1996). Sub-samples of dried soil material from the incubation experiment and from the original soils were homogenised in a ball mill. Thereafter, total C and total N were determined using gas chromatography after combustion, using a Carlo Vario Max CN analyser (Elementar, Hanau). After incubation, particulate organic matter (POM) was determined in two fractions (63–400 μm and >400 μm) by size separation through wet sieving (Magid and Kjaergaard, 2001). A sub-sample of approximately 40 g of soil (on an oven-dry basis) was dispersed with 40 ml NaCl-solution (5%) by 30 min horizontal shaking at 200 rev $min^{-1}$ and washed with distilled deionised water over the sieves. POM was burned to ash at 550 °C and dry matter was recalculated on an ash free basis. As an approximation, the maize derived POM of the respective fraction (63–400 μm, >400 μm) was estimated by subtracting the POM in the control treatment from the POM in the maize treatment. Total POM was defined as the sum of the POM from both determined fractions.

### 2.4. Determination of soil microbial properties

Microbial biomass C and biomass N in the soil before and after incubation were estimated by fumigation–extraction (Brookes et al., 1985; Vance et al., 1987). Briefly, one portion of 10 g (on an oven-dry basis) soil was immediately fumigated for 24 h at 25 °C with ethanol-free $CHCl_3$. Following fumigant removal, the sample was extracted with 40 ml 0.5 M $K_2SO_4$ by 30 min horizontal shaking at 200 rev $min^{-1}$ and filtered through a folded filter paper (Schleicher and Schuell 595 1/2). The non-fumigated 10 g portion was extracted similarly at the time when fumigation commenced. Organic C in the extracts was measured as $CO_2$ by infrared absorption after combustion at 850 °C using a Dimatoc 100 automatic analyser (Dimatec, Essen, Germany).

Table 1
Soil physical and chemical properties of the original and the washed soils before the incubation experiment

| Property | Soil A | Soil B | Soil A | Soil B | n |
|---|---|---|---|---|---|
| | Original | | After washing | | |
| $EC_e$ (dS $m^{-1}$) | 1.2 a | 42.0 b | 0.6 a | 0.9 a | 3 |
| pH ($CaCl_2$) | 4.67 a | 5.35 b | 5.11 c | 5.53 d | 3 |
| CEC ($mmol_c$ $kg^{-1}$ soil) | 132 a | 184 b | 124 a | 175 b | 3 |
| SAR | 0.1 a | 20.4 b | 0.3 a | 0.3 a | 3 |
| Sand (%) | 73 a | 76 a | 76 a | 76 a | 3 |
| Clay (%) | 8 a | 13 b | 7 a | 8 a | 3 |
| Bulk density (g $cm^{-3}$) | 0.82 a | 0.92 a | ND | ND | 5 |
| Organic C (mg $g^{-1}$ soil) | 16.8 a | 36.2 b | 15.8 a | 32.2 c | 4 |
| Extractable C (μg $g^{-1}$ soil) | 110 a | 302 b | 98 c | 190 d | 4 |
| Organic C-to-total N | 11.9 a | 13.4 b | 11.4 a | 15.6 c | 4 |
| Total N (mg $g^{-1}$ soil) | 1.4 a | 2.7 b | 1.4 a | 2.1 c | 4 |

Values with different letters within a row show means with significant differences (HSD=honestly significant difference, Tukey/Kramer, $p<0.05$, ND=not determined; $EC_e$=electrical conductivity; CEC=cation exchange capacity; SAR=sodium adsorption ratio).

Table 2
Soil microbial properties of the maize straw used in the incubation experiment, as well as of the original soils and the washed soils before the incubation experiment

| Property | Maize straw (mean±SD) | n | Soil A | Soil B | Soil A | Soil B | n |
|---|---|---|---|---|---|---|---|
| | | | Original | | After washing | | |
| Microbial biomass C (μg $g^{-1}$ soil) | 39,300±10,200 | 7 | 386 a | 87 b | 439 c | 118 b | 4 |
| Microbial biomass N (μg $g^{-1}$ soil) | 2300±800 | 6 | 64 a | 12 b | 67 a | 11 b | 4 |
| Microbial biomass C-to-biomass N | 19.4±5.0 | 6 | 6.0 a | 7.3 a | 6.5 a | 11.1 b | 4 |
| Microbial biomass C-to-organic C (%) | 9.7±2.1 | 7 | 2.29 a | 0.24 b | 2.78 c | 0.37 b | 4 |
| Ergosterol (μg $g^{-1}$ soil) | ND | | 1.03 a | 0.17 b | 1.28 a | 0.14 b | 3 |
| Ergosterol-to-microbial biomass C (%) | ND | | 0.27 a | 0.15 a | 0.30 a | 0.12 b | 3 |
| Muramic acid (μg $g^{-1}$ soil) | 59±3 | 5 | 47 a | 75 b | ND | ND | 5 |
| Glucosamine (μg $g^{-1}$ soil) | 5561±502 | 5 | 883 a | 1035 a | ND | ND | 5 |
| Galactosamine (μg $g^{-1}$ soil) | n.d. | 5 | 399 a | 510 b | ND | ND | 5 |
| Mannosamine (μg $g^{-1}$ soil) | 548±47 | 5 | 193 a | 214 b | ND | ND | 5 |
| Fungi-to-bacteria ratio | 18.6±1.4 | 5 | 3.8 a | 2.8 b | ND | ND | 5 |

Values with different letters show means with significant differences between original and washed soil (SD=standard deviation, n.d.=not detected, HSD=honestly significant difference, Tukey/Kramer, $p<0.05$, ND=not determined).

Microbial biomass C was $=E_C/k_{EC}$, where $E_C$=(organic C extracted from fumigated soils)−(organic C extracted from non-fumigated soils) and $k_{EC}=0.45$ (Wu et al., 1990). Total N in the extracts was measured by chemoluminescence detection after combustion at 850 °C, using a Dima-N automatic analyser. Microbial biomass N was $=E_N/k_{EC}$, where $E_N$=(total N extracted from fumigated soils)−(total N extracted from non-fumigated soils) and $k_{EN}=0.54$ (Brookes et al., 1985; Joergensen and Mueller, 1996).

The microbial biomass C and N content in the maize straw was determined in the same way, including a pre-extraction step to remove soluble compounds (Mueller et al., 1992). Briefly, two portions of 10 g of dry maize straw were extracted with 200 ml 0.05 M $K_2SO_4$ by 30 min horizontal shaking at 200 rev min−1 and filtered through a folded filter paper (Schleicher and Schuell 595 1/2). One portion of the extracted straw including the filter was fumigated immediately and the other portion extracted again with another 200 ml 0.05 M $K_2SO_4$ at the time fumigation commenced. In the pre-extracts and extracts, organic C and total N were measured as described above. In the $K_2SO_4$ extracts of all non-fumigated soil samples, $NO_3^-$–N and $NH_4^+$–N were determined using segmented flow analysis. The $K_2SO_4$ extractable organic N was calculated as the difference between total extractable N minus inorganic N ($NO_3^-$–N and $NH_4^+$–N).

The ergosterol content in the original and washed soils and after incubation was measured according to Djajakirana et al.

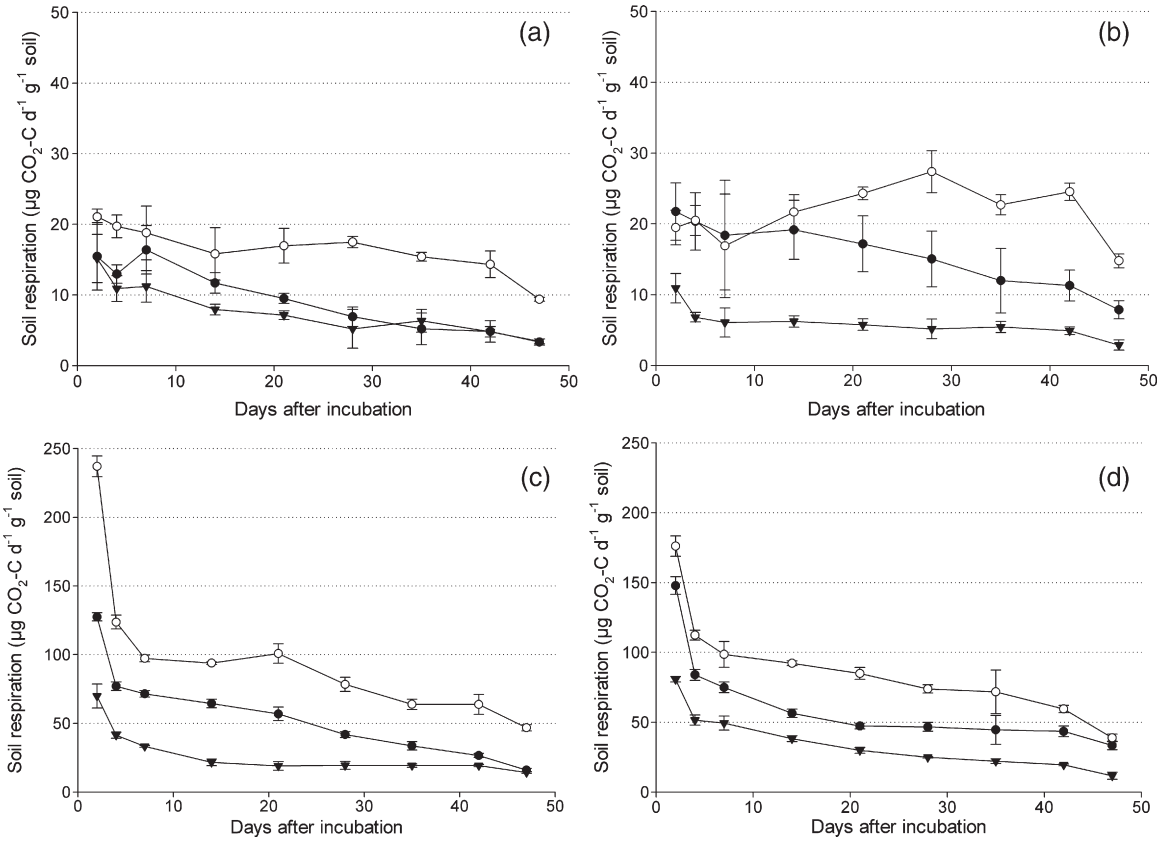


Fig. 1. Soil respiration rate (µg $CO_2$–C $d^{-1}$ $g^{-1}$ soil) of the control soils A (a) and B (b), and after maize addition to soil A (c) and soil B (d), without salt addition (○), with 15 mg NaCl $g^{-1}$ soil (●), and with 50 mg NaCl $g^{-1}$ soil (▼) during the incubation experiment (47 days at 25 °C). Error bars indicate standard deviation ($n$=4).

(1996). Briefly, 2 g moist soil were extracted with 100 ml ethanol for 30 min by oscillating shaking at 250 rev $min^{-1}$ and then filtered (Whatman GF/A). Ergosterol determination was done using a reversed-phase HPLC analysis at 26 °C, using a 125 mm×4 mm Sphereclone 5 µm ODS II column with a Phenomenex guard column (4 mm×3 mm). Chromatography was performed isocratically with methanol (100%) and a resolution of detection of 282 nm (Dionex UVD 170 S).

In the original soils, in the maize straw, and in the POM, the four amino sugars muramic acid, glucosamine, galactosamine, and mannosamine were determined according to Appuhn et al. (2004). Sub-samples of 0.3 g (on an oven-dry basis) maize straw and POM were rewetted with 10 ml 6 M HCl for 17.5 h. Thereafter, the samples were hydrolysed at 105 °C for 3 h. From the original and washed soils, moist sub-samples of 0.5 g (on an oven-dry basis) were immediately hydrolysed with 10 ml 6 M HCl for 6 h at 105 °C. After filtration (Whatman GF/A), a 0.5 ml (0.3 ml for the maize straw) aliquot of each hydrolysate was evaporated at 40 °C to dryness. The residue was rinsed with 0.5 ml distilled deionised water, evaporated to dryness again, then taken up in 1 ml water, and centrifuged at 5000 ×$g$. Samples were stored at − 18 °C until HPLC measurement. For HPLC measurement, 5 µl of the sample and 20 µl OPA-reagent were mixed in the sample loop and injected onto the column (Phenomenex C18 Hypersil 5 µm ODS, 125 mm×4 mm and Phenomenex security guard cartridge C18, ODS, 4 mm×2 mm). Detection of the fluorometric emission of amino sugar derivatives took place at a wavelength of 445 nm, with 340 nm as the excitation wavelength (Agilent FLD 1100 G 1321 A, set at the amplification PMT 16).

Assuming that muramic acid in the samples was solely of bacterial origin and that bacterial cells contained muramic acid and glucosamine in the same amounts on a molar basis, we were able to calculate the fungi-to-bacteria ratio. Further assumptions were that the C content in bacterial and fungal cells was 46% (Jenkinson, 1988) and that bacterial cells contain on average 10.3 mg muramic acid $g^{-1}$ dry matter and fungal cells contain on average 49 mg glucosamine $g^{-1}$ dry matter (Appuhn and Joergensen, 2006). The amount of bacterial C in the samples was calculated by multiplying the content of muramic acid by 45. Fungal C content in the samples was calculated by multiplying fungal glucosamine (total glucosamine − bacterial glucosamine) by 9. Fungal glucosamine was calculated by subtracting bacterial glucosamine from total glucosamine (mol glucosamine − mol muramic acid) × 179.17 (molecular weight of glucosamine). The fungi-to-bacteria ratio was thus fungal C divided by bacterial C. Microbial residue C in the maize straw was estimated by subtracting the microbial biomass C from the total microbial C which has been calculated from the amino sugars (fungal C + bacterial C).

### 2.5. Statistical analysis

Results presented in the tables and figures are arithmetic means and presented on an oven-dry basis (about 24 h at

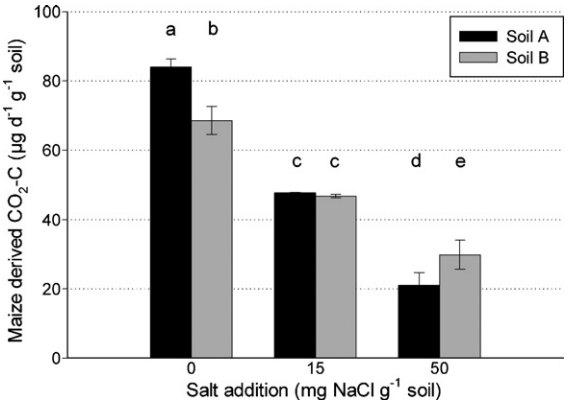


Fig. 2. Maize derived C evolved as $CO_2$ (µg $CO_2$–C $d^{-1}$ $g^{-1}$ soil) during the 47 day incubation experiment. Values with different letters show means (±SD) with significant differences (Tukey/Kramer, $p<0.05$, $n=4$).

105 °C). Three-way analyses of variance of the data were carried out using StatView 5.0 (SAS Inst. Inc., Cary, NC, USA) and the Tukey/Kramer test (HSD) was computed to separate means.

## 3. Results

### 3.1. Soil and substrate properties

Electrical conductivity ($EC_e$) was 35 times higher in soil B than in soil A, showing very high salinisation (Table 1). After washing, both soils were on a similar level. The two soils did not differ in soil texture substantially, with sand mainly derived from New Red Sandstone making up approximately 75% of the organic matter free soil. Soil A had a slightly lower pH in comparison to B. The slightly higher cation exchange capacity in soil B corresponded to the higher soil organic C content in comparison with soil A. With this higher content of organic C, soil B had an elevated C-to-N ratio in comparison to A, which was even more pronounced after washing the soils, due to the fact that high amounts of extractable compounds were removed by washing, reflected by the decrease of the extractable C content. Microbial biomass C, biomass N, ergosterol, the ergosterol-to-microbial biomass C ratio, and the microbial biomass C-to-soil organic C ratio were all lower in soil B in comparison to A (Table 2). On the other hand, the C-to-N ratio of the microbial biomass was higher in soil B. The four amino sugars detected were on a higher level in soil B in comparison with soil A. The total amino sugar content in soil B was higher than in A, which corresponds to the higher soil organic C content in soil B. Glucosamine made up 58% (soil A) and 56% (soil B) of the detected amino sugars. However, statistically it was not significantly different in the two soils. The fungi-to-bacteria ratio of the two original soils calculated from the glucosamine and muramic acid content was significantly different, with soil A having a value of 3.8 and soil B having a ratio of 2.8.

After fumigation–extraction with a pre-extraction step, the maize straw contained 39.3 mg microbial biomass C $g^{-1}$ dry matter and 2.3 mg microbial biomass N $g^{-1}$ dry matter, resulting in a C-to-N ratio of 19 (Table 2). The total amino sugar content in the maize straw was more than 6 mg $g^{-1}$ dry matter and was strongly dominated by glucosamine (90%). Muramic acid made up 1% of the amino sugars and mannosamine contributed 9% to the amino sugars detected. The fungi-to-bacteria ratio calculated from muramic acid and glucosamine was 18.6. Approximately 25% of the microbial C detected in the maize straw was microbial residue C, indicating that the major proportion of the amino sugars were of biomass origin.

### 3.2. Organic matter decomposition and N-mineralisation

Soil respiration decreased in all treatments during the experiment (Fig. 1). It only slightly decreased in the control treatments (Fig. 1a, b) and was on a lower level in comparison to the maize treatments. In the treatments with addition of 0 and 15 mg NaCl, soil respiration remained on a higher level in soil B in comparison with soil A. Soil respiration was at a maximum after 2 days in all maize treatments and reached a plateau after approximately 30 days, with a further slight decrease after almost 7 weeks (Fig. 1c, d). Soil A showed a higher initial soil respiration for the 'no salt' treatment, while the initial respiration was higher in soil B for the treatment with addition of 15 mg NaCl. The initial respiration was similar in both soils after addition of 50 mg NaCl. Maize derived $CO_2$–C (µg $d^{-1}$ $g^{-1}$ soil) was higher in soil A for the treatment without salt addition, but higher in soil B where 50 mg NaCl $g^{-1}$ soil were added (Fig. 2).

There was no significant difference in maize derived total POM after incubation between the salt treatments and between the two soils. However, maize derived C recovered in the POM showed a trend towards an increase with elevated salinity, especially in the fraction >400 µm in soil A (Fig. 3). The maize derived C recovered in the POM fraction of 63 to 400 µm size was slightly higher in soil B in comparison with soil A, but was on a similar level irrespective of the salt content. The strong variability in the recovery of maize C after incubation is due to high variability of the POM data.

Extractable C and extractable organic N contents increased with elevated salt content in soil A (Table 3). This was more

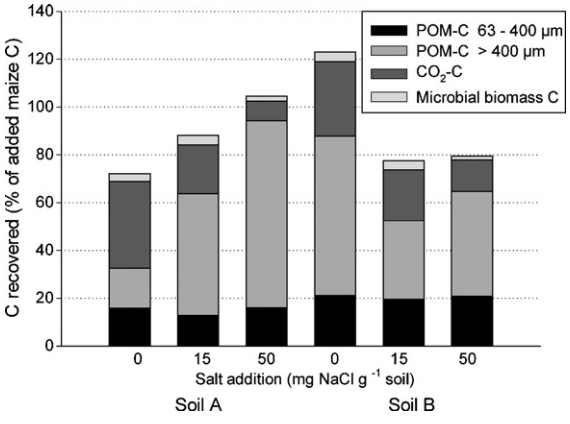


Fig. 3. Maize C recovered (% of added maize C) as POM in the fractions 63–400 µm and >400 µm, as microbial biomass C, and as $CO_2$–C after incubation for 47 days at 25 °C.

Table 3
Extractable C, extractable organic and inorganic nitrogen pools in the soil after 47 days of incubation

| Treatment and soil | Salt addition (mg NaCl $g^{-1}$ soil) | Extractable C (μg $g^{-1}$ soil) | Extractable organic N (μg $g^{-1}$ soil) | $NH_4^+$–N (μg $g^{-1}$ soil) |
|---|---|---|---|---|
| *Control soil A* | | | | |
| | 0 | 127 a | 17 a | 7.2 a |
| | 15 | 181 a | 70 b | 24.4 b |
| | 50 | 154 a | 60 c | 18.7 b |
| *Control soil B* | | | | |
| | 0 | 296 a | 28 a | 2.7 a |
| | 15 | 221 a | 19 b | 12.1 b |
| | 50 | 216 a | 19 b | 29.6 c |
| *Soil A with maize straw* | | | | |
| | 0 | 143 a | 18 a | 1.4 a |
| | 15 | 209 a | 48 b | 10.8 b |
| | 50 | 325 b | 75 c | 17.2 b |
| *Soil B with maize straw* | | | | |
| | 0 | 263 a | 27 a | 0.7 a |
| | 15 | 191 a | 17 b | 0.6 a |
| | 50 | 263 a | 30 a | 2.0 a |
| *Analysis of variance* | | | | |
| Treatment | | 9.6** | 0.2 | 84.3**** |
| Salt Addition | | 5.1* | 249.3**** | 53.6**** |
| Soil | | 23.2**** | 769.1**** | 22.2**** |
| $T \times SA$ | | 12.7**** | 63.4**** | 8.6*** |
| $T \times S$ | | 12.9** | 6.3* | 9.0** |
| $SA \times S$ | | 19.2**** | 389.9**** | 6.8** |
| Residual | | 36 | 36 | 36 |

Values with different letters within a column show means with significant differences between the salt treatments for the respective soil ($p<0.05$; Tukey/Kramer HSD-test). The lower part of the table shows $F$-values from the analysis of variance; degrees of freedom: treatment (1), salt addition (2), soil (1); treatment (control or maize), salt addition (0, 15 or 50 mg NaCl $g^{-1}$ soil), soil (A or B).
*$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$.

pronounced after maize addition. In soil B this trend could not be observed and the content of extractable C rather showed a decline with increasing salt content. The extractable organic N content in soil A was on a higher level for the salt treatments in comparison with soil B. After incubation, $NH_4^+$–N strongly dominated the inorganic N fraction and almost no nitrate was detected. It significantly increased with elevated salt content. Maize addition decreased the inorganic N content drastically but values were lower in soil B in comparison to soil A.

### 3.3. Microbial properties after incubation

Microbial biomass C, microbial biomass N, and the ergosterol content all decreased with increasing salt content and were on a higher level in the maize treatments in comparison with the control (Tables 4 and 6). Microbial biomass and ergosterol were higher in soil A than in soil B (Table 4). The ergosterol-to-microbial biomass C ratio was slightly higher after maize addition and showed a weak trend towards an increase with elevated salt content (Table 5). However, the fungi-to-bacteria ratio in the POM showed no trend with increasing salinity (Table 6). It varied between 0.2 and 0.5, demonstrating bacterial dominance, and significantly decreased after maize addition (Table 6). Microbial biomass N decreased more markedly with increasing salt content in soil A than in soil B (Table 4, Fig. 4). In contrast, the decrease of microbial biomass C with increasing salinity was more pronounced in soil B in comparison to A. This difference was reflected by an increase in the C-to-N ratio of the microbial biomass with increasing salt content in the control soil A and a decrease in the control soil B (Table 5), which was not statistically significant. The metabolic quotient $qCO_2$ was significantly different between the two soils, but there was no correlation with the salt content (Table 5).

## 4. Discussion

### 4.1. Impact of salinity on mineralisation processes

The accumulation of organic matter in the saline soil (soil B), as also observed by Rasul et al. (2006) in the same area, showed the detrimental effect of salinity on the degradation processes of

Table 4
Microbial biomass C (MBC) and N (MBN) and ergosterol content in the soil after 47 days of incubation

| Treatment and soil | Salt addition (mg NaCl $g^{-1}$ soil) | MBC (μg $g^{-1}$ soil) | MBN (μg $g^{-1}$ soil) | Ergosterol (μg $g^{-1}$ soil) |
|---|---|---|---|---|
| *Control soil A* | | | | |
| | 0 | 295 a | 59 a | 1.31 a |
| | 15 | 192 b | 33 b | 1.36 a |
| | 50 | 120 b | 20 b | 0.99 a |
| *Control soil B* | | | | |
| | 0 | 171 a | 22 a | 0.67 a |
| | 15 | 86 a | 18 a | 0.32 a |
| | 50 | 61 b | 15 a | 0.25 a |
| *Soil A with maize straw* | | | | |
| | 0 | 569 a | 110 a | 4.54 a |
| | 15 | 543 a | 86 a | 7.71 b |
| | 50 | 299 b | 56 b | 3.04 a |
| *Soil B with maize straw* | | | | |
| | 0 | 513 a | 67 a | 5.62 a |
| | 15 | 423 a | 53 b | 4.53 ab |
| | 50 | 193 b | 36 c | 2.35 b |
| *Analysis of variance* | | | | |
| Treatment | | 290.5**** | 251.0**** | 110.3**** |
| Salt Addition | | 65.0**** | 55.3**** | 10.7*** |
| Soil | | 37.8**** | 99.1**** | 7.8** |
| $T \times SA$ | | 13.9**** | 5.5** | 5.9** |
| $T \times S$ | | 0.0 | 7.0* | 0.0 |
| $SA \times S$ | | 0.5 | 10.2*** | 4.5* |
| Residual | | 36 | 36 | 36 |

Values with different letters within a column show means with treatment-specific significant differences ($p<0.05$; Tukey/Kramer HSD-test). The lower part of the table shows $F$-values from the analysis of variance; degrees of freedom: treatment (1), salt addition (2), soil (1); treatment (control or maize), salt addition (0, 15 or 50 mg NaCl $g^{-1}$ soil), soil (A or B).
*$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$.

Table 5
Microbial biomass C-to-N ratio (MBC-to-N), ergosterol-to-microbial biomass C ratio (ergosterol-to-MBC), and the metabolic quotient $qCO_2$ in the soil after 47 days of incubation

| Treatment and soil | Salt addition (mg NaCl $g^{-1}$ soil) | MBC-to-N | Ergosterol-to-MBC (%) | $qCO_2$ (mg $CO_2$–C $g^{-1}$ MBC $d^{-1}$) |
|---|---|---|---|---|
| *Control soil A* | | | | |
| | 0 | 5.0 a | 0.45 a | 44 a |
| | 15 | 6.7 a | 1.09 a | 26 a |
| | 50 | 7.9 a | 0.86 a | 44 a |
| *Control soil B* | | | | |
| | 0 | 7.4 a | 0.38 a | 140 a |
| | 15 | 4.8 a | 0.45 a | 158 a |
| | 50 | 4.1 a | 0.53 a | 77 a |
| *Soil A with maize straw* | | | | |
| | 0 | 5.2 a | 0.79 a | |
| | 15 | 6.4 a | 1.40 a | |
| | 50 | 5.6 a | 1.04 a | |
| *Soil B with maize straw* | | | | |
| | 0 | 7.6 a | 1.10 a | |
| | 15 | 8.0 a | 1.08 a | |
| | 50 | 5.5 a | 1.20 a | |
| *Analysis of variance* | | | | |
| Treatment | | 0.5 | 20.2**** | |
| Salt Addition | | 0.6 | 3.3* | 0.7 |
| Soil | | 0.1 | 2.0 | 12.4** |
| $T \times SA$ | | 1.0 | 0.1 | |
| $T \times S$ | | 4.2* | 3.5 | |
| $SA \times S$ | | 5.0* | 2.7 | 1.4 |
| Residual | | 36 | 36 | 18 |

Values with similar letters within the column of a treatment show that means are not significantly different ($p<0.05$; Tukey/Kramer HSD-test). The lower part of the table shows $F$-values from the analysis of variance; degrees of freedom: treatment (1), salt addition (2), soil (1); treatment (control or maize), salt addition (0, 15 or 50 mg NaCl $g^{-1}$ soil), soil (A or B).
*$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$

organic matter, underlining our first hypothesis. It is the result of slower transformation of organic substrates, due to a smaller microbial biomass and a restricted microbial metabolism (Mamilov et al., 2004). Sardinha et al. (2003) observed a similar trend for soil microbial properties in the same area, but they were not able to detect any differences in soil organic C content with increasing salinity. Rietz and Haynes (2003) observed a decreased soil organic matter decomposition in saline soils and concluded that this was balanced by a lower organic matter input due to reduced plant growth. In the present study the high organic matter content in the saline soil B is attributed to small scale variability of the organic matter content and to its accumulation before salinity occurred.

The reduced $CO_2$-evolution rate with an increase in salinity in our incubation experiment revealed a decrease in C-mineralisation, which is in line with the findings of others (e.g. Pankhurst et al., 2001; Pathak and Rao, 1998). However, in our study, the $CO_2$-evolution did not further decrease from 15 to 50 mg NaCl $g^{-1}$ soil in the control soil A, remaining on a level of 6 to 8 μg $CO_2$–C $g^{-1}$ soil, providing evidence of tolerance to osmotic stress of the remaining microbes. Nevertheless, even at very high salinity with electrical conductivity of 97 dS $m^{-1}$, significant $CO_2$-evolution was observed (Pathak and Rao, 1998), indicating that mineralisation occurs even under strongly saline conditions.

The increasing amounts of recovered maize C in the POM fraction >400 μm in soil A further supported our hypothesis, showing that the added substrate remained less decomposed with increasing salinity. In soil B on the other hand, the maize derived C in the POM fraction >400 μm was highest where no salt was applied and lower than the respective amounts in soil A when 15 or 50 mg NaCl were added. This indicates that decomposition under salinity is stronger when microorganisms have previously been prone to salinity, which might be the result of eco-physiological adjustment to the specific environmental conditions (Dilly, 2001).

We observed that inorganic N in the salt treatments was almost exclusively $NH_4^+$–N, as observed by others (Pathak and Rao, 1998), revealing that nitrification was inhibited (Laura, 1974). In the present study all measured biological indices decreased with increasing salinity, but inorganic N content increased. Laura (1974) argued that the increase of inorganic N

Table 6
Mean fungi-to-bacteria ratio of the particulate organic matter (POM) after 47 days of incubation calculated from the amino sugar content

| Treatment and soil | Salt addition (mg NaCl $g^{-1}$ soil) | Fungi-to-bacteria ratio | | |
|---|---|---|---|---|
| | | Particulate organic matter (POM) | | |
| | | 63–400 μm | >400 μm | Total (>63 μm) |
| *Control soil A* | | | | |
| | 0 | 0.42 a | 0.37 a | 0.38 a |
| | 15 | 0.44 a | 0.41 a | 0.42 a |
| | 50 | 0.44 a | 0.45 a | 0.44 a |
| *Control soil B* | | | | |
| | 0 | 0.36 a | 0.48 a | 0.43 a |
| | 15 | 0.35 a | 0.47 a | 0.41 a |
| | 50 | 0.43 a | 0.51 a | 0.45 a |
| *Soil A with maize straw* | | | | |
| | 0 | 0.26 a | 0.34 a | 0.29 a |
| | 15 | 0.25 a | 0.32 a | 0.28 a |
| | 50 | 0.34 a | 0.36 a | 0.35 a |
| *Soil B with maize straw* | | | | |
| | 0 | 0.48 a | 0.28 a | 0.37 a |
| | 15 | 0.40 a | 0.20 a | 0.30 a |
| | 50 | 0.46 a | 0.26 a | 0.34 a |
| *Analysis of variance* | | | | |
| Treatment | | 1.4 | 38.5**** | 18.1*** |
| Salt Addition | | 1.0 | 1.1 | 1.1 |
| Soil | | 2.4 | 0.2 | 1.1 |
| $T \times SA$ | | 0.2 | 0.6 | 0.4 |
| $T \times S$ | | 10.1** | 11.5** | 0.1 |
| $SA \times S$ | | 0.2 | 0.5 | 0.8 |
| Residual | | 36 | 36 | 36 |

Values with similar letters within the column of a treatment show that means are not significantly different ($p<0.05$; Tukey/Kramer HSD-test). The lower part of the table shows $F$-values from the analysis of variance; degrees of freedom: treatment (1), salt addition (2), soil (1); treatment (control or maize), salt addition (0, 15 or 50 mg NaCl $g^{-1}$ soil), soil (A or B).
*$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$.

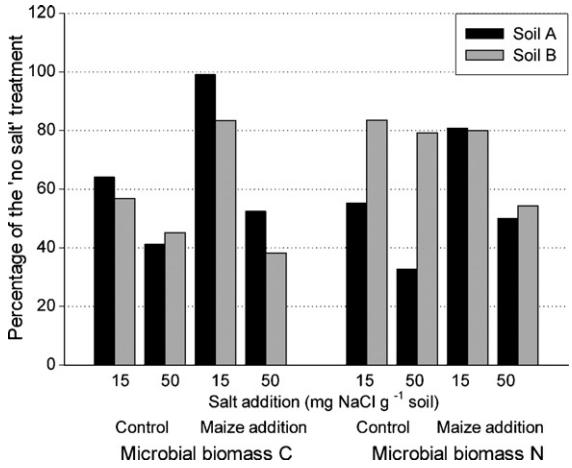


Fig. 4. The decrease of microbial biomass C and N in relation to the respective 'no salt' treatment and in response to 15 mg NaCl $g^{-1}$ soil and 50 mg NaCl $g^{-1}$ soil during the 47 days of incubation ($n$=4).

at high salinity is mediated mainly chemically and that biological and biochemical N-mineralisation only occurs to a limited extent. In our experiment, the amounts of $NH_4^+$–N mobilized in the salt treatments are almost always smaller than the N lost from the microbial biomass. We assume that autolysis effects after cell death in relation to elevated salinity might thus account for most of the increase in inorganic N observed at high soil salinity in our study. Nevertheless, the observed microbial activity even under strong salinity suggests that biological N-mineralisation occurred even at high osmotic pressure (Pathak and Rao, 1998).

### 4.2. Response of the microbial communities to salinity

Salinity strongly inhibited microbial biomass, and resulted in a decrease of substrate decomposition in our experiment, as also observed by others (Rietz and Haynes, 2003; Sardinha et al., 2003), supporting our hypothesis. The addition of maize straw increased the microbial biomass content substantially. It reduced the negative effect of salinity especially in the treatment with 15 mg NaCl $g^{-1}$ soil where the microbial biomass was not different from the treatment without salt. There is still a relatively high microbial biomass C content in both of our soils after treatment with 50 mg NaCl $g^{-1}$ soil and addition of maize in comparison to the control, underlining the positive effect of substrate addition.

The slight trend towards an increase of the ergosterol-to-microbial biomass C ratio in the soil with increasing salinity observed in our experiment is in contrast to the findings of Sardinha et al. (2003). This might reflect a shift of the microbial community towards those organisms adapted to high osmotic pressure (e.g. fungi with a higher ergosterol content) and thus does not necessarily reflect a shift towards fungi. In addition, it cannot be totally excluded that ergosterol from dead fungi was measured (Zhao et al., 2005), as degradation of ergosterol is hampered especially in saline conditions. Our hypothesis that strong salinity results in a stronger decrease of fungi is thus not supported by our data. The addition of maize straw increased the ergosterol content substantially. Estimates of the microbial biomass and the measurement of amino sugars on the maize straw, showed a high content of microorganisms dominated by fungi. The increase in microbial biomass and ergosterol after maize straw addition was thus at least partly due to the addition of living organisms, especially fungi. However, after incubation, the fungi-to-bacteria ratio of the POM was much lower than on the original maize straw and in the original soils, revealing bacterial dominance in the decomposer community on the substrate. Furthermore, after maize addition, the bacterial dominance on the POM was even more pronounced than in the control soils. This is even more astonishing, as salinity had no effect on the fungi-to-bacteria ratio in the POM.

A wider C-to-N ratio of the microbial biomass in soil B is evidence that the microbial community is different from that in soil A. This might reflect a shift in the microbial community structure or a change in cell morphology (Sardinha et al., 2003), as observed for bacterial populations under salt stress (Schimel et al., 1989; Zahran, 1997). The trend of a decreasing microbial biomass C-to-N ratio with increasing salinity as observed in soil B, demonstrates the adaptation mechanisms of cells to osmotic stress, resulting in a selective accumulation of incorporated ions to counteract the osmotic pressure (Killham, 1994).

The high energy demand for the selective exclusion of $Na^+$, as described by Killham (1994) for single cells of different species of microbes, was documented on a community level by an increased $qCO_2$ in our experiment. It was significantly different between the two soils, but not between the salt treatments, highlighting that the respective soil microbial community was in a similar physiological state, regardless of the salt concentration during the 7 weeks of our experiment. The lower amounts of extractable organic C and N in the salt treatments of soil B in comparison to soil A might be attributed to the higher physiological activity of the microbial community in soil B. It ultimately results in a stronger incorporation of this substrate into microbial biomass and microbial metabolites, indicating a less efficient substrate use by the microbial community (Rietz and Haynes, 2003). However, it has to be kept in mind that the $qCO_2$ is influenced by the quality and amount of respirable C-substrates (Sparling, 1997), which might differ between the two investigated soils.

During this short-term incubation, no detectable adaptation of the microbial community to increased osmotic pressure occurred. On the other hand, the physiological difference between soil A and soil B might reflect a long-term adaptation of the microbial community in soil B, which has been prone to soil salinity for decades. The detected physiological differences fit the implications of the adaptation mechanisms described by Killham (1994). This finding supports the concept that long-term stress to a microbial community will result in a new dynamic equilibrium (Van Bruggen and Semenov, 2000). The present results support our hypothesis that the microbial community previously prone to salinity has adapted to it. However, the adaptation mechanism with a higher physiological activity will result in increased soil respiration and thus C mineralisation, and in immobilisation of N in microbial metabolites. Our fourth hypothesis that adaptation of the microorganisms results in increased mineralisation can thus be only partly verified.

## 5. Conclusions

Salinity has detrimental effects on soil microbial communities, resulting in a decrease of soil respiration, microbial biomass, and related decomposition and mineralisation processes. It is not necessarily associated with a shift towards bacteria. Long-term salinisation results in a microbial community that uses substrate less efficiently, indicated by a high metabolic quotient. This reflects energy rich adaptation mechanisms of the microbial community to the increased osmotic pressure, which has previously been described only for single cells and microbial populations. Amendment of organic matter improves soil quality under saline conditions and counteracts the negative effects of salt, because soil microorganisms profit from a higher substrate availability and can more easily cope with high salinity. It also leads to a spatial differentiation of the microbial community in the soil, with bacteria dominating the surface of the substrate. Salinity seems not to influence this differentiation process.

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