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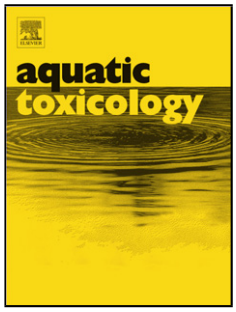

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Author: Felix G. Sauer Mirco Bundschuh Jochen P. Zubrod Ralf B. Schäfer Kristie Thompson Ben J. Kefford

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Effects of salinity on leaf breakdown: dryland salinity versus salinity from a coalmine

Felix G. Sauer[1,2], Mirco Bundschuh[2,3], Jochen P. Zubrod[2], Ralf B. Schäfer[2], Kristie Thompson[4], Ben J. Kefford[1]

[1]Institute for Applied Ecology, University of Canberra, ACT 2601, Australia

[2]Institute for Environmental Sciences, University of Koblenz-Landau, Fortstrasse 7, 76829 Landau, Germany

[3]Department of Aquatic Sciences and Assessment, Swedish University of Agriculture Sciences, Uppsala, Sweden

[4]National Research Centre for Environmental Toxicology, The University of Queensland, 39 Kessels Road, Coopers Plains, Queensland 4108, Australia

Corresponding author:

Email address: Ben.Kefford@canberra.edu.au (Ben J. Kefford)

Highlights

(1) Increasing salinity decreased microbial leaf breakdown rates above ≈ 500 µS/cm

(2) Microbial leaf breakdown peaked at an intermediate salinity (≈ 500 µS/cm)

(3) Ionic composition of salinity alters the amount of microbial leaf breakdown

Abstract

Salinization of freshwater ecosystems as a result of human activities represents a global threat for ecosystems' integrity. Whether different sources of salinity with their differing ionic compositions lead to variable effects in ecosystem functioning is unknown. Therefore, the present study assessed the impact of dryland- (50 µS/cm to 11,000 µS/cm) and coalmine-induced (100 µS/cm to 2,400 µS/cm) salinization on the leaf litter breakdown, with focus on microorganisms as main decomposer, in two catchments in New South Wales, Australia. The breakdown of *Eucalyptus camaldulensis* leaves decreased with increasing salinity by up to a factor of three. Coalmine salinity, which is characterised by a higher share of bicarbonates, had a slightly but consistently higher breakdown rate at a given salinity relative to dryland salinity, which is characterised by ionic proportions similar to sea water. Complementary laboratory experiments supported the stimulatory impact of sodium bicarbonates on leaf breakdown when compared to sodium chloride or artificial sea salt. Furthermore, microbial inoculum from a high salinity site (11,000 µS/cm) yielded lower leaf breakdown at lower salinity relative to inoculum from a low salinity site (50 µS/cm). Conversely, inoculum from the high salinity site was less sensitive towards increasing salinity levels relative to inoculum from the low salinity site. The effects of the different inoculum were the same regardless of salt source (sodium bicarbonate, sodium chloride and artificial sea salt). Finally, the microorganism-mediated leaf litter breakdown was most efficient at intermediate salinity levels (≈ 500 µS/cm). The present study thus points to severe implications of increasing salinity intensities on the ecosystem function of leaf litter breakdown, while the underlying processes need further scrutiny.

Keywords: Microorganisms; Freshwater; Salinization; Leaf breakdown; Major ions

1. Introduction

The integrity of freshwater ecosystems, including the functions these systems provide, are increasingly threatened by anthropogenic activities within their catchment (Millennium Ecosystem Assessment 2005). Salinization of freshwater systems is an increasingly recognized disturbance, which can, for instance, be induced by large-scale irrigation, clearing of natural vegetation and mining (Cañedo-Argüelles et al. 2016), and is reinforced by climate change (John et al. 2005; Hughes 2003). Characterizing salinization by an increase in the electric conductivity (EC) (Williams and Sherwood 1994) or other measures of total ion concentrations does, however, not fully reflect its complexity: the composition of major ions can differ tremendously with their sources. This composition of ions ultimately modulates the consequences for aquatic ecosystems (Cañedo-Argüelles et al. 2016).

The anthropogenic salinization of freshwater ecosystems in Australia is mainly driven by two factors. Firstly, a removal of the natural deep-rooted native vegetation leads to a rise of saline ground water towards the surface (i.e., dryland salinity) (Williams 2001). Dryland salinity is typically dominated by sodium and chloride as the major ions with the proportions of other ions being usually similar to sea water (Rengasamy 2006). Secondly, saline effluents from coal mining and coal seam gas extraction have a more variable ionic composition, with many effluents exhibiting high levels of bicarbonate, an ion which is found in very low concentrations in seawater and is rarely found at high levels in other natural aquatic ecosystems except in chalk streams (Berrie 1992). This diversity of salt contamination in Australia – but also most other parts of the world, calls for the development of a more mechanistic- and process-orientated understanding of the factors triggering the salt-induced effects in ecosystems and particularly the functions or processes these systems provide (Cañedo-Argüelles et al. 2013).

A key ecosystem process in freshwater ecosystems is the breakdown of allochthonous organic matter such as leaf litter in fine particular organic matter finally fuelling the heterotrophic food web (Webster et al. 1999; Bundschuh and McKie 2016). This fundamentally important function is, however, sensitive towards increasing levels of salinity as empirically underpinned by field studies in southeast Australia (Schäfer et al. 2012) and the Appalachian in the US (Fritz et al. 2010) as well as a stream mesocosm experiment in Spain (Cañedo-Argüelles et al. 2014). However, the role of the ionic composition and thus the source of salinization for this ecosystem process have not been assessed but might be of fundamental importance for the development of a target orientated risk mitigation strategy by environmental managers.

The present study assessed litter breakdown by the contribution of microorganisms and invertebrates in stream ecosystems subjected to a variable intensity of dryland- and coalmine-induced salinization in two Australian catchments. Complementary laboratory experiments were conducted to verify the role of the ionic composition for the effects observed under field conditions thereby excluding potentially confounding factors. The main objectives of this study were to characterize potentially inhibitory implications of salinity induced by two different sources (i.e., dryland and coalmine) in the leaf litter breakdown in the field and to determine the role of the ionic composition for the impact in this variable.

2. Material and Methods

2.1. Location and study design of the field study

The field study was conducted at 22 sites in New South Wales, Australia, (see supplementary material S1, for locations) between March and early May 2014. Fifteen sites were located in the Murrumbidgee River Catchment and seven sites in the Georges River Catchment. Parts of the Murrumbidgee Catchment are strongly affected by dryland salinity (Jolly et al. 2001) whereas the George River Catchment is impacted by a coalmine effluent released into Brennans Creek by West Cliff Colliery. The sites were selected to exhibit a gradient of EC ranging from ≈ 50 µS/cm to ≈ 11,000 µS/cm and ≈ 2,400 µS/cm for dryland and coalmine salinity, respectively.

2.2. Field study

Breakdown was determined using leaves of *Eucalyptus camaldulensis* Dehnh. (River Red Gum), a common riparian tree in Australia, collected in February 2014 from a single tree on the banks of the Yass River (34°55'19.73"S, 149°10'49.04"E) and oven dried at 60 °C to a constant mass. Approximately 2.5 ± 0.1 g dried leaves were put into each fine (mesh size < 1 mm, cylinder length ≈ 15cm) and coarse mesh bags (size ≈ 5 mm, bag size ≈ 20 × 15 cm), with five replicate bags of each type at each site to determine the microorganism mediated and total leaf litter breakdown realised by microorganisms and invertebrates, respectively (Gessner and Chauvet 2002). The fine bags had an additional section with one leaf of approximately 0.2 g dry mass used to quantify the fungal biomass. Small stones were added to each bag to keep the bags submerged. After five weeks the bags were retrieved, carefully washed in tap water to remove adhering debris and macroinvertebrates, dried at 60°C and weighed to determine the remaining leaf mass in each bag. A subsample of the dried leaves of each bag was used to determine its phosphorus concentration (see details below). The additional leaf for fungal biomass quantification was freeze-dried and stored frozen until analysis.

At each site, temperature loggers (Hobo Data loggers, Onset, Pocasset, USA) were deployed and used to compute the breakdown rate per sum of degree days (dday) (Petersen and Cummins 1974). For each replicate the breakdown rate *k* was calculated:

$$k_{leaves\ per\ i} = \frac{-\ln\left(\frac{S_i(t)}{S_i(0)}\right)}{\sum_{j=1}^{t} \overline{T_i}\ (j)}$$

where $S_i(t)$ and $S_i(0)$ are the final and initial mass, respectively, at site *i*, *t* is the total number of deployment days and $\overline{T}$ is the mean temperature for a day *j*.

At seven sites, where salinity was expected to be high, both temperature and electric conductivity (EC in µS/cm at 25°C, hereafter µS/cm) were logged (Hobo Data loggers, Onset, Pocasset, USA). At all sites, water parameters were measured in the field with a multi-meter (Horiba, Kyoto, Japan) during the deployment and retrieval of leaves. These measurements included temperature (°C), EC, dissolved oxygen (mg/L), oxygen saturation (%), turbidity (NTU) and pH. Additionally, alkalinity ($CaCO_3$ in mg/L) was determined by sulphur acid titration (APHA 1999) on site. Filtered (0.2 µm) water samples were taken and stored frozen to quantify the composition of the major ions by ionic chromatography.

At ten sites, where we could not exclude the occurrence of pesticide pollution based on upstream landuse, passive samplers (i.e., chemcatcher) (Kingston et al. 2000) and passive flow monitoring (PFM) were used to determine pesticides exposure (O'Brien et al. 2011). After 5 weeks the samplers were retrieved from the streams and stored frozen until further analyses (ENTOX, Brisbane, Australia see for details (O'Brien et al. 2014). The pesticide concentrations were subsequently normalised to their ecotoxicological potential for algae (i.e., *Pseudokirchneriella subcapitata*) and invertebrates (i.e., *Daphnia magna*) using the toxic units (TU) approach:

$$TU = \frac{c_i}{LC50_{i,j}}$$

where *c* is the concentration of the toxic compound *i* and *j* the benchmark organism (Sprague 1970). The toxicity data were taken from (Malaj et al. 2014).

2.3. Lab study

The laboratory-based experiments aimed at determining the role of the ionic composition for the salinity-induced effects in the microorganism-mediated leaf litter breakdown. At the same time a cause-response relationship between the degree of salinization and the effect size was

targeted. Therefore, dried *E. camaldulensis* leaves (as above) were in a first step conditioned (= colonized by microorganisms) in fine mesh bags at the site exhibiting either the lowest (≈ 50 µS/cm) or the highest (≈ 11,000 µS/cm) EC within the Murrumbidgee Catchment (i.e., from dryland salinity). After 14 days of conditioning, four marked field-conditioned leaves were used as microbial inoculum to condition another 2.0 ± 0.1 g of dried but unconditioned *E. camaldulensis* leaves per experimental unit (= replicate) plus one marked unconditioned leaf for the determination of the fungal biomass. The replicates consisted of a plastic container filled with 600 ml deionized water adjusted to 100 µS cm^{-1} with artificial sea salt (Ocean Nature) – serving also as control (n = 15). Six additional factor combinations (n = 5) were established by three salt types, namely sodium chloride (NaCl), artificial sea salt (Sea) or sodium bicarbonate ($NaHCO_3$), at two EC levels each (i.e., 1,000 and 10,000 µS/cm). Thereby this experiment took advantage of a multifactorial design, with 2 sources of microbial inoculum, 3 types of salt in combination with 2 levels of EC (3 for sea salt at 100 (the control), 1,000 and 10,000 µS/cm) resulting in 14 treatments. The experiment lasted four weeks with a water exchange (using the same treatments as before for each replicate) after two weeks and was performed in a climate chamber with a 12:12h day:night cycle under continuous aeration. The temperature cycle was set at 20°C during nights and increased linearly to 25°C at the middle of the day and subsequently decreased to 20°C. At the termination of the experiment the field-conditioned (inoculation) leaves were removed from each replicate and the leaf mass loss over the experimental duration was determined based on the dry mass of the leaves that were not conditioned. As in the field study, a subsample of the dried leaf mass was used to determine its phosphorus concentration (see details below) and the leaf material used for the quantification of the fungal biomass was freeze-dried and stored frozen until analysis.

Following the same approach, a second laboratory experiment was conducted to determine alterations in the microbial leaf breakdown in response to a lower range of salinity to establish a cause-effect relationship. Electrical conductivity was adjusted to 50, 100, 200, 500, 1000 µS/cm (n = 5) using sea salt as the only salt type and inoculum generated exclusively from the low salinity dryland site. Fungal biomass and phosphorus were not measured during this experiment.

2.4. Leaching

Additional experiments were conducted in the laboratory as well as in the field to estimate the proportion of the mass loss attributed to (abiotic) leaching and thus neither to the activity of microorganisms nor invertebrates over 96 hours (Lush and Hynes 1973; Quinn et al. 2000). In

the laboratory, 2 g dried eucalyptus leaves were subjected for 96 hours to the same treatments as described above (n = 5). In the field, 2.5 g leaf material were deployed in fine bags at four sites (n = 10): two sites were located upstream and two sites downstream of the coalmine's discharge. After four days, the leaf mass loss was determined based on the reduction in the leaves dry weight.

2.5. Quantification of Ergosterol

Following Gessner and Schmitt (1996) fungal biomass was estimated using ergosterol as proxy. Ergosterol was extracted in alkaline methanol from the freeze-dried leaf samples and purified by a solid-phase extraction (SPE: Sep-Pak® Vac RC tC18 500mg sorbent, Waters, Milford, MA, USA). Subsequently, ergosterol was quantified using high-performance liquid chromatography (HPLC, 1200 Series, Agilent Technologies, Santa Clara, CA, USA) with a Li-Chrospher 100 RP 18-5 µm column (250.0 mm x 4.6 mm, particle size 5 µm, CS-Chromatographie Service, Langerwehe, Germany) by measuring the absorbance at 282 nm. **2.6. Determination of phosphorus**

Approximately 0.5 g of dried leaf per sample in the field and per experimental unit in the laboratory experiment was digested with 1 ml of hydrochloric acid and 2 ml of nitric acid in 30 ml vessels on a hot block at 100°C for 2 hours. After cooling, the digests were diluted with deionized water to a final volume of 30 ml (Esslemont et al. 2000). Phosphorus was determined with an ACP-AES (Alternating Current Plasma- Atomic Emission Spectrometry by the Australian Laboratory Services; ALS, Canberra, Australia).

2.7. Data analysis

The data generated during the field study were averaged for each variable (water parameter and response variables) per site. Subsequently, linear models were used to analyse the correlation between salinity – expressed as log-transformed EC – and the response variables, namely macroinvertebrate- or microorganism-mediated leaf breakdown as well as the ergosterol and phosphorus concentration of the conditioned leaves. Moreover, linear models were utilized to detect potential relationships between the response variables and the further water parameters (oxygen saturation, pH, total alkalinity and turbidity). The statistical significance of the two sources of salinization (dryland and coalmine) and the EC in general was judged using analysis of covariance (ANCOVA). Three-way analysis of variance (ANOVA) was employed for the laboratory-generated data to determine the statistical significance of EC, salt type and sources of microorganisms (and their interactions) for the response variables. The means of the leaching experiments and the second laboratory experiment examining microbial leaf breakdown across

a lower range of salinity were compared through a one-way ANOVA. In case of statistical significance, a post-hoc Tukey-test was used to compare the different treatments. Before conducting ANOVAs or ANCOVAs, data were inspected visually as suggested by most authors (e.g., Zuur et al. 2009) for outliers, normality and homogeneity of variances. However, Bartlett (for homogeneity of variances) and Shapiro-Wilks (for normality) tests were conducted complementarily to check the assumptions for parametric testing. Confidence intervals (95%) for the means of Figure 3 and 4 were calculated in accordance with Altman et al. (2000). R version 3.2.1 was used for figures and statistics (R Core Team 2015) and the piper diagram was created with the software GW chart (Winston 2000). The term "significant(ly)" is solely used in the sense of statistical ($p < 0.05$) but not necessarily biological significance.

3. Results

3.1. Physiochemical water parameters

The five sites downstream the coalmine were strongly dominated by sodium and carbonates, whereas the dryland sites had a more balanced composition of anions and cations (Figure 1). Two sites upstream the coalmine discharge had ionic composition similar to the dryland sites. The ionic composition of the sodium bicarbonate treatments in the lab experiment was similar to the sites downstream of the coalmine whereas the composition of sea salt was comparable to most of the dryland sites. The pH across all sites ranged from 6.31 to 7.24 (see supplementary material S2). The pesticide toxicity ranged from 1.3×10^{-5} to 4.4×10^{-3} TUs for *P. subcapitata* and 7.6×10^{-9} to 1.8×10^{-3} TUs for *D. magna* (see supplementary material Table S3).

3.2. Relationship between environmental variables and the response variables in the field

Linear models showed a significant decrease of microbial leaf breakdown with increasing salinity (Figure 2), whereas sites affected by coalmine effluents showed higher breakdown rates relative to dryland sites at a given salinity - although not significantly ($p = 0.052$). A similar but also not significant ($p = 0.16$) relationship was observed for the leaf mass loss in coarse mesh bags (Figure S1). Almost no difference in leaf breakdown was observed between fine and coarse bags at most of the sites. The fungal biomass as indicated by the ergosterol concentration was not related to salinity. The ergosterol concentration, however, was significantly greater at the coalmine sites (mean: 33 mg/kg) than at the dryland sites (mean: 10 mg/kg) (see supplementary material Figure S2). The phosphorus concentration in the leaves showed no significant correlation with salinity ($p = 0.75$) or sources of salinization ($p = 0.84$) (see supplementary material Figure S3). None of the further water quality parameters correlated with

the response variables microbial leaf breakdown rate, ergosterol or phosphorus concentration in the leaves (r^2 values between 0.01 and 0.07, for details see supplementary material Table S5). The leaching mass loss in field ranged from 23.5% to 26.3%, but did not reveal a significant difference between up- and downstream sites of the coalmine's discharge (supplementary material Figure S4).

3.3. Lab studies

The factors, salt type, EC and source of the inoculum significantly affected leaf mass loss (all *p*-values < 0.001, Figure 3). There were also significant interactions between the source of the inoculum and EC, and source of the inoculum and the salt type (both *p*-values < 0.001) but no significant (p = 0.36) three-way interaction. In general, greater leaf mass loss was observed in presence of sodium bicarbonate at a given salinity. Considering the source of the inoculum separately, no significant effect of EC was observed when microorganisms were sampled from the high salinity site ($p = 0.41$). Yet with the inoculum from the low salinity site, leaf mass loss decreased significantly at high EC levels (i.e., 10,000 µS/cm; $p < 0.001$). This pattern may explain the significant interaction in terms of the source of the inoculum with salt type and EC. Ergosterol did not show any significant response to EC, salt types or the source of inoculum (see supplementary material Figure S6). The phosphorus concentration in the leaves was significantly higher when inoculum from the low salinity site was used ($p < 0.001$) and decreased with increasing salinity ($p < 0.001$). The salt type had no statistically significant influence on the phosphorus concentration (see supplementary material Figure S5).

The second laboratory experiment, which was established to determine alterations in the microbial leaf breakdown in response to a lower range of salinity detected significant differences among the treatments ($p = 0.018$). Hereby, the microbial breakdown peaked at an intermediate EC level of 500 µS/cm (Figure 4), where a Tukey-test also revealed a significant greater mass loss in comparison with the 0 and 50 µS/cm treatments.

Finally, there was statistically significantly greater leaching related leaf mass loss over 96 hours in presence of sodium bicarbonate relative to the other salt types at a given EC (≈ 3-4%) (supplementary material Figure S7).

4. Discussion

The present study found a negative correlation between salinity and the leaf litter breakdown for both dryland- and coalmine-induced salinity. Macroinvertebrate-mediated leaf litter breakdown played a minor role at all sites pointing to microorganisms as the main decomposers,

which is likely due to the warm region of the study area what enhances the contribution of microorganisms processing to leaf breakdown (Irons et al. 1994). Consequently, the present study links increasing salinity to decreasing microorganism-mediated leaf litter breakdown (Figure 2) and is thus in agreement with other field studies (Fritz et al. 2010; Schäfer et al. 2012). Various environmental factors like water quality parameters (e.g., temperature, dissolved oxygen; Hagen et al. 2006) and a range of different anthropogenic stressors can influence the microorganism-mediated leaf litter breakdown (Young et al. 2008; Tank et al. 2010) potentially hampering the establishment of a cause and effect relationship between salinization and leaf litter breakdown. However, as none of the measured water quality parameters correlated with the response variable these parameters may be excluded as a source for confounding effects (supplementary material Table S5). Anthropogenic stressors with the highest potential to modify the leaf litter breakdown in our study area are pesticides (Schäfer et al. 2007) and metals (Clarlisle and Clements 2005): The effect-normalised concentrations of pesticides measured at the sampling sites were largely below threshold (Sprague 1970) $TU_{\textit{D. magna}}$ and $TU_{\textit{P.subcapita}}$ of 1×10^{-3} (Table S3). Consequently, the implications of pesticides in the leaf litter breakdown of the present study are likely low (Schäfer et al. 2012). Also the metal concentrations are regularly measured in the study area, particularly downstream of the coalmine's discharge in the George River, but the maximal concentrations (Cu ≈ 7 µg/l, Al ≈ 750 µg/l, Zn ≈ 35 µg/l, Ni ≈ 100 µg/l measured by EPA (2012)) are up to thousandfold lower than those shown to reduce leaf breakdown (Bermingham et al. 1996; Sridhar et al. 2001). Thus, it may be suggested that water quality parameters, pesticides and metals had only a limited impact on the outcome of the present study and salinity is likely the major cause for the reduced microorganism-mediated leaf litter breakdown rates, which was also indicated by the laboratory experiment of the present study (Figure 3).

In further support of this hypothesis Cañedo-Argüelles et al. (2014) uncovered a reduced leaf litter breakdown as a consequence of repeated salt pulses at intensities of some 10,000 µS/cm, while leaf-associated fungal biomass was partly stimulated. This increase in fungal biomass is, however, not consistent with field studies showing either negative (Schäfer et al. 2012) or no consistent impact in this endpoint (Fritz et al. 2010) with increasing salinity.

Similarly, the fungal biomass (estimated using ergosterol as proxy) was not consistently affected by salinity during both the field and the laboratory experiment of the present study (Figure S2 and S6). Ca^{2+} might stimulate fungi (Chamier and Dixon 1983) but there was no significant correlation between Ca^{2+} in mg/L and ergosterol ($r^2 = 0.12$, $= 0.24$) and the sites

with the highest Ca^{2+} concentration had the lowest breakdown rate (see Table S1 and S2). Also during the laboratory experiment, no stimulating effect of Ca^{2+} was observed since the sea salt treatment of 10,000 µS/cm had much higher amount of Ca^{2+} in comparison with the other treatments but revealed no greater leaf breakdown rates. In general, the amount of ergosterol was relatively low in this study which may be explained by a replacement of the aquatic hyphomycetes through bacterial communities as it was shown in response to increasing temperature by Foucreau et al. (2016). These insights suggest that the implications of salinization on the microorganism-mediated leaf litter breakdown might also cause a shift in leaf associated microbial community (Zubrod et al. 2015) and their metabolic activity (Ward and Brock 1978; Tyree et al. 2016). In support of this assumption, the inoculum originating from the high salinity site had – irrespective of the intensity of salinity – a lower efficiency to break down leaf material relative to inoculum from the low salinity site (Figure 3), suggesting the loss of microbes efficiently degrading leaf litter during the communities' adaptation to highly saline environments. Besides the leaf litter degradation efficiency also the microorganisms' ability to assimilate phosphorus from the surrounding medium was reduced with increasing salinity and was higher for microorganisms from low salinity sites (see Figure 3 and supplementary material Figure S5) (Qualls and Richardson 2000). These differences in turn point to a reduced activity of the leaf associated microbial community indirectly supporting the assumption of a shift in the community composition.

The leaf litter breakdown by microorganisms did not follow a negative monotonic trend with increasing salinity. Six of seven sites with the highest breakdown rate in the field were in a range between 180 and 410 µS/cm with lower breakdown at the lowest saline sites (≈ 50 µS/cm) (Figure 2) and during the second laboratory experiment the leaf breakdown efficiency peaked at an intermediate sea salt dominated salinity (i.e., 500 µS/cm; Figure 4) – although only statistically significant towards the 0 and 50 µS/cm treatments. When, however, combining these results, they indicate that the leaf breakdown rate rather followed an inverted `U´ shaped concentration-response relationship with an optimum largely between 200 and 500 µS/cm. This outcome is in accordance with a number of studies suggesting optimal conditions in such a range of salinity (200-500 µS/cm) for macroinvertebrate diversity (Kefford et al. 2011) and, freshwater fishes (Boeuf and Payan 2001), insects (Hassell et al. 2006) and gastropods (Kefford and Nugegoda 2005) and indicates the importance of a minimum of ions for the performance of microorganisms.

It remains, however, unclear whether the different strengths in the relationship between the microorganism-mediated leaf litter breakdown and salinity in dryland- and coalmine-impacted systems can be explained by the composition of major ions. Since the ionic composition can substantially modify the threshold-levels in terms of EC for the salt types' specific toxicity for macroinvertebrates but also fish (Mount et al. 1997), the first laboratory experiment addressed the impact of three salt types, namely sodium chloride, artificial sea salt or sodium bicarbonate, at two intensities (i.e., 1,000 and 10,000 µS/cm) on the microorganism-mediated leaf litter breakdown. In contrast to Kefford et al. (2004) reporting substantial difference in the ecotoxicological potential of artificial sea salt and sodium chloride for freshwater invertebrates, no difference in microbial leaf breakdown was observed here (Figure 3). Nonetheless, a stimulating effect of sodium bicarbonate was uncovered in the laboratory experiment (Figure 3) and is partly supported by the field data (Figure 2). This pattern may be explained by sodium bicarbonate buffering against the acidic microenvironment generated by the microorganisms' activity, which could hamper the further breakdown of leaf litter, indirectly stimulating microorganism-mediated leaf litter breakdown (Brock et al. 1985). The slightly higher leaf mass loss as a consequence of leaching (≈ 3-4%) in presence of sodium bicarbonate (Figure S7) may also have partly contributed to the higher breakdown rates in the laboratory study but cannot entirely explain this effect (Figure 3). Although the mechanisms for the stimulatory effect induced by sodium bicarbonate have not been unravelled during the present study, the data clearly point to an ionic composition specific impact in the ecosystem process of leaf litter breakdown, which deserves further investigation.

5. Conclusion

The present study clearly shows that salinity is a threat for the integrity of freshwater ecosystems, which is presumably modulated by the ionic composition of the salinity. At the same time, the underlying processes leading to an impairment of the leaf litter breakdown is not fully understood: studies addressing the structural and functional response of leaf-associated microorganisms would greatly enhance our understanding on the mechanisms driving the response on a functional level.

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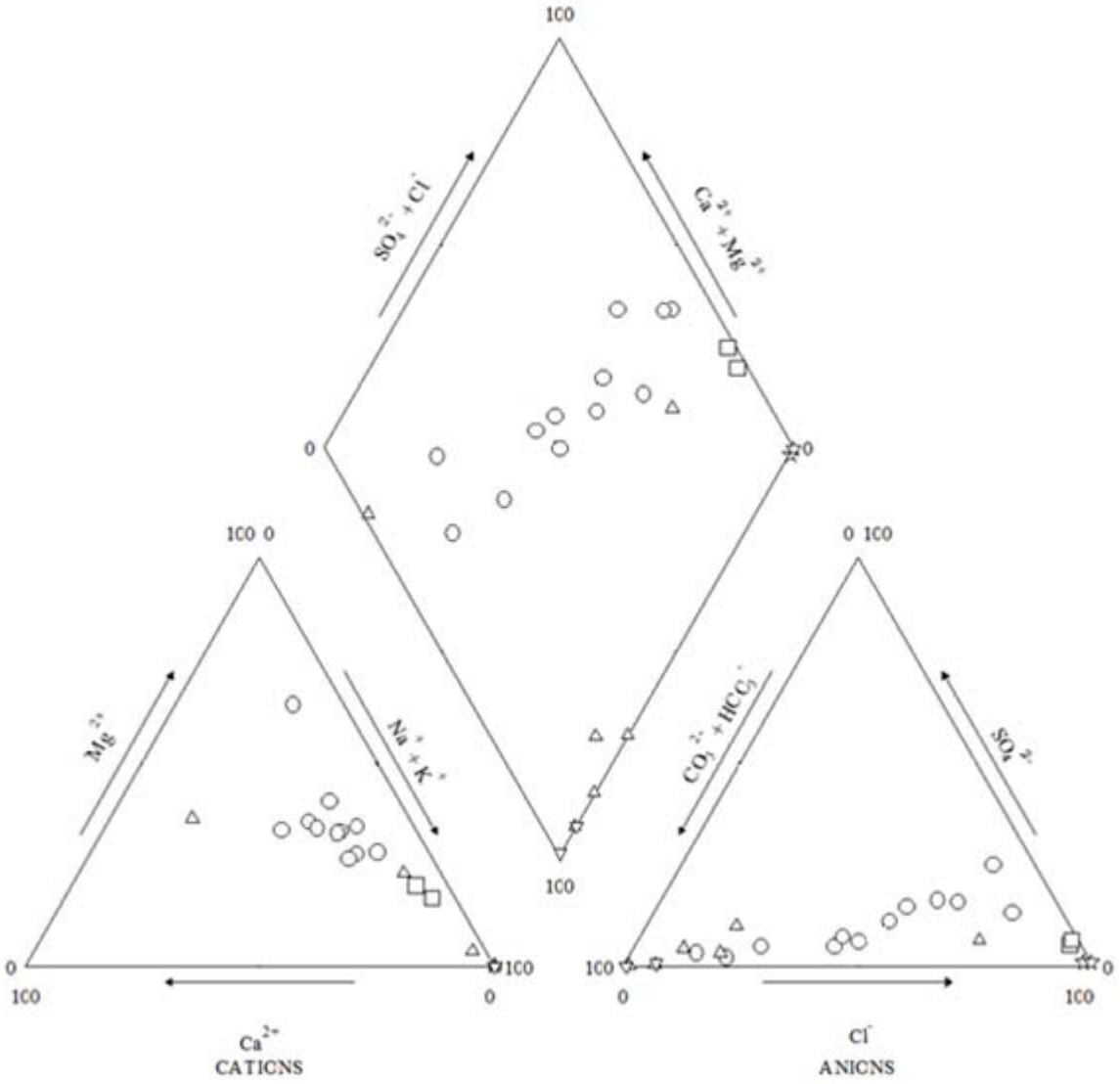

Figure 1: Piper diagram of the ionic composition from the field sites (dryland: circle; coalmine: triangle) and from lab experiments (sea salt: square; NaCl: star; $NaHCO_3$: inverted triangle). The proportions are calculated as percentage of milliequivalents based on Tables S2 and S4 (see supplementary material). The diagram in the centre describes the ionic composition of the different sites/treatments by combining the composition of cations (diagram on the left) and anions (diagram on the right).

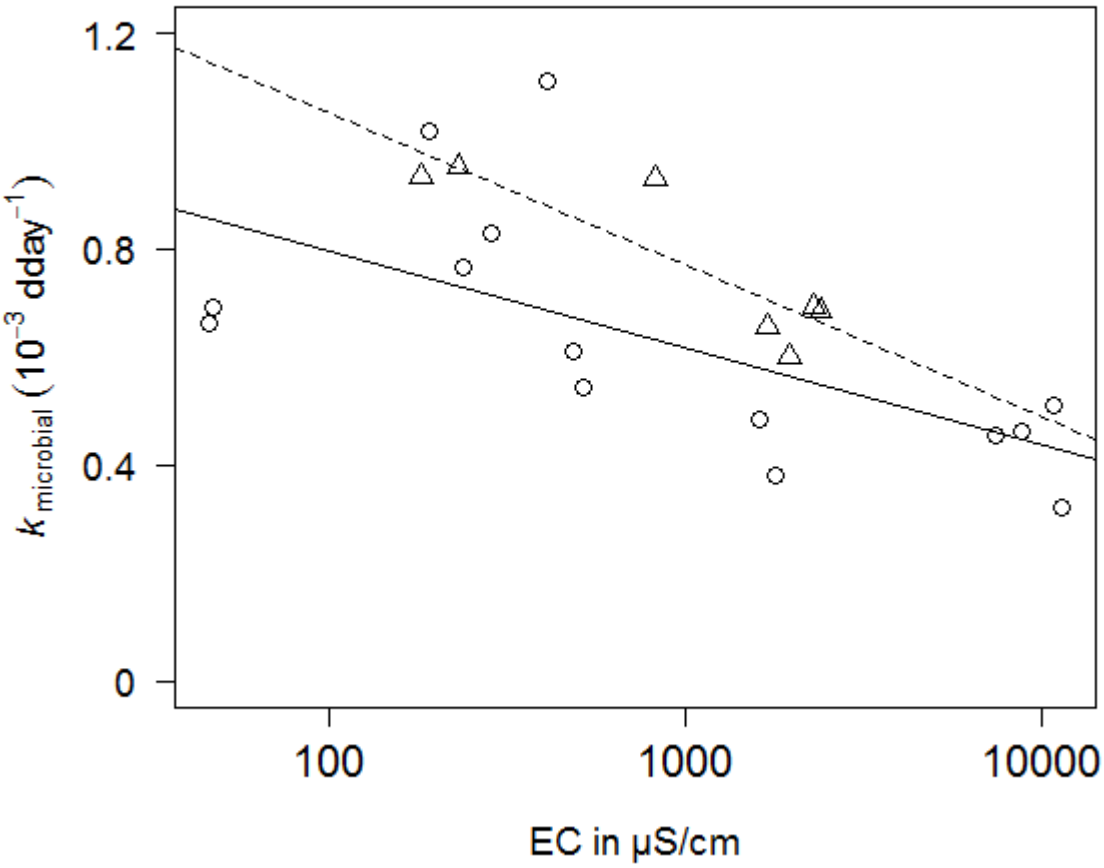

Figure 2: Correlation between salinity (with logarithmic scaling) and microbial leaf breakdown rate ($k_{microbial}$) for dryland (circle) and coalmine (triangle) salinity. Log EC explains 36% of the variation at the dryland sites (the continuous line) and 72% of variation for the coalmine sites (the dashed line).

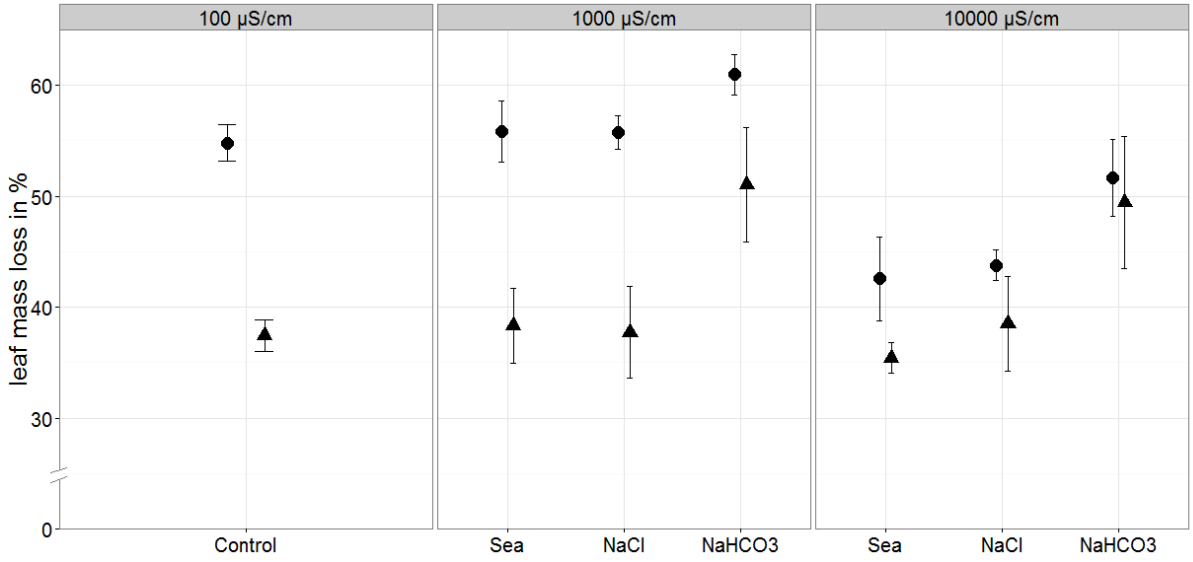

Figure 3: Mean microorganism-mediated leaf mass loss in per cent (± 95% confidence interval) under well-controlled laboratory conditions after 28 days in presence of different types of salt at 1,000 and 10,000 µS/cm. The control was composed of sea salt at 100 µS/cm. As microbial inoculum colonized leaves sampled from a site with low (circles) and high (triangles) levels of salinity were used.

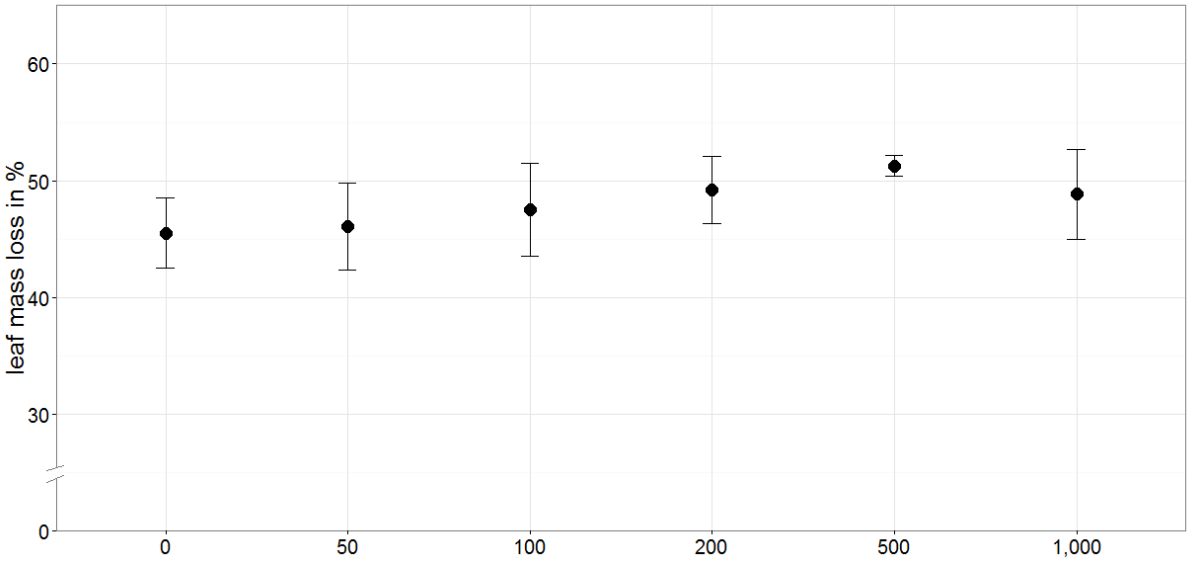

Figure 4: Mean microorganism-mediated leaf mass loss in per cent (± 95% confidence interval) at increasing levels of EC using sea salt as salt type. Deionized water was used for the treatment with 0 µS cm^{-1}. The inoculum was generated at the low salinity site.