

Silica decouples fungal growth and litter decomposition without changing responses to climate warming and N enrichment

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Abstract. Ongoing global changes, such as climate warming and increasing supply of reactive nitrogen (N), are expected to affect essential ecosystem processes such as the decomposition of plant litter. Determining the influence of environmental heterogeneity on the magnitude of these effects remains an important task, with silicon (Si) availability being a notable component of this heterogeneity, especially for grasses. We conducted an outdoor enclosure experiment to test if increased Si supply to a widespread foundation species (*Phragmites australis*) alters the effect of climate warming and excess N supply on litter decomposition by curbing fungal decomposers. Consistent with expectations, Si supply during plant growth reduced fungal biomass in decomposing leaf blades by 50%, an effect that was doubled by excess external N supply. These strong impacts, however, did not directly translate to reduced litter decomposition or associated changes in nutrient dynamics. Instead, plant tissue-specific effects determined the influence of Si, N, and elevated temperature on litter mass loss. Specifically, Si accelerated the decomposition of leaf sheaths, warming enhanced leaf-sheath and leaf-blade decomposition, and N decreased the decomposition of culm litter, in line with expectations based on differences in litter chemistry. Thus, despite highly detrimental effects of Si and N on fungal decomposers, compensation by other members of the microbial community could dampen the realized impact of these global-change factors on the decomposition of plant litter in the future.

Key words: ecosystem processes; experimental warming; fungal biomass; global change; mesocosm; nitrogen deposition; *Phragmites australis*; silica cycle.

INTRODUCTION

Multiple factors of global change are simultaneously confronting ecosystems worldwide. Although model predictions vary in magnitude, warming trends observed in the recent past are consistently projected to continue in the future (IPCC 2007, Blunden et al. 2011), leading to rising temperatures not only in the atmosphere and oceans but also in inland waters such as lakes, rivers and wetlands (Adrian et al. 2009, Jeppesen et al. 2010). Anthropogenic activities are also altering the global availability of several biologically important elements. Best known is the massive conversion of atmospheric molecular nitrogen (N₂) to biologically available forms, mainly as a result of industrial processes and growth of nitrogen-fixing leguminous plants in agriculture (Galloway et al. 2008). This has doubled the natural global N

fixation rate, resulting in widespread increases in N loading of surface waters (Vörösmarty et al. 2010), sometimes altering species distributions, food web interactions, and rates of biogeochemical cycling, which has marked consequences for the structure and functioning of aquatic ecosystems (Jeppesen et al. 2010).

An accumulation of evidence suggests that climate warming and nitrogen deposition will also impact the decomposition of plant litter, an essential ecosystem process in terrestrial, wetland, and many aquatic ecosystems. Warming tends to accelerate decomposition, whereas increases in external nitrogen supply can accelerate or retard decomposition, depending on litter chemistry (Knorr et al. 2005, Hobbie et al. 2012). However, the magnitude of global change effects on ecosystem processes will not be uniform across all environments. An intrinsic feature of ecosystems is the spatial heterogeneity in the distribution of mineral resources available to primary producers. This heterogeneity occurs at multiple scales, including the landscape scale. As a result, plants have evolved a huge number of adaptations for tolerating, or capitalizing on, environ-

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mental heterogeneity. Determining how these adaptations at the plant and plant tissue level scale up to influence responses of ecosystems to global change remains an important task.

Silicon, the second most abundant constituent in the Earth's crust, is spatially distributed in a heterogeneous fashion and could play an important role in the sensitivity of decomposition to global environmental changes, such as climate warming and excess nitrogen deposition. In particular, ecosystems dominated by silicon-accumulating grasses typically cycle large amounts of silicon via mobilization, root uptake, and litter fall (Cornelis et al. 2010). Silicon also assumes special importance in the defense of plants, particularly against large ungulate herbivores (McNaughton and Tarrant 1983, McNaughton et al. 1985), herbivorous rodents (Soininen et al. 2013), and insects (Massey and Hartley 2009), and there is also evidence that silica in plants is effective against fungal infestation (Fauteux et al. 2005). Deterrent properties of Si are likely to remain effective after plant death. Therefore, increases in silica concentrations in plant tissues could impede fungal decomposers in ways similar to Si effects on live plant tissue utilization by fungal pathogens. By limiting growth of fungal decomposers, silicon accumulation in plants could dampen the impact of warming and nitrogen deposition on decomposition, as the impact of these global-change factors are manifest to a large extent through effects on microbial metabolism.

Responses of plants to environmental heterogeneity occur mainly at the level of individual plant tissues. That is, plant tissues (e.g., leaf blades, leaf sheaths, and stems) differ in their elemental composition and also in the extent to which those tissues are plastic in response to environmental heterogeneity. Consequently, the impact of global change on decomposition is likely to vary across individual plant tissues, either due to tissue-specific differences in Si content and plasticity in the tendency to accumulate Si, or because silicon availability during plant growth modifies other aspects of tissue chemistry besides Si concentrations (e.g., C:N:P ratios, cellulose and phenol contents [Schaller et al. 2012a, b, 2013]). However, the magnitude of these effects and, in particular, their interactions with N availability and climate warming as forecast by climate-change scenarios, is virtually unknown at present. Si heterogeneity is likely to play an increasingly important role in ecosystem responses to global change in the future as natural weathering rates are altered (Struyf et al. 2010) and human population growth demands increased harvesting of Si-accumulating crops (Casey et al. 2004), thus changing Si mobilization and transport across landscapes and effectively resulting in heterogeneous Si deposition in wetlands and lakes (Struyf et al. 2010).

The present experiment was designed to test how variation in silicon availability affects the impact of climate warming and excess nitrogen supply on plant

litter decomposition in a littoral freshwater marsh. We hypothesized that (1) warming will accelerate decomposition by stimulating microbial metabolism, and that (2) the impact of increased nitrogen supply on decomposition would depend on the recalcitrance of the plant litter. Importantly, we hypothesized that (3) by negatively affecting fungal decomposers, silicon will retard decomposition and dampen the effects of other factors of global change; and that (4) silica effects would be manifest at a plant-tissue-specific level with the strongest effect on decomposition occurring in the tissue with the highest initial silica content. By examining fungal biomass, nutrient immobilization, and decomposition, this study tested how plant responses to environmental heterogeneity in Si supply during plant growth could modify response of decomposition to two main factors of environmental change: climate warming and increased nitrogen deposition.

MATERIAL AND METHODS

Study site

The study was carried out in an outdoor climate-change facility located in a littoral marsh on the eastern shore of Lake Hallwil, Switzerland (47°17'14.00" N, 8°13'18.53" E), a eutrophic, postglacial, moraine lake with a surface area of 10.2 km² and an average depth of 28.6 m. Emergent wetland vegetation composed almost exclusively of *Phragmites australis* (Cav.) Trin. ex Steud. formed a band up to 20 m wide along portions of the shoreline. Maximum water depth at the lakeward marsh margin was about 1 m. Water chemistry and other characteristics of the lake, its littoral *Phragmites* marsh, as well as the design and functioning of the facility, are given in Hines et al. (2013).

Experimental design

To determine the impact of warming, excessive external nitrogen supply, and litter silica concentration on decomposition, fungal biomass accrual, and nutrient immobilization in decomposing litter, we conducted a 2 × 2 × 3 factorial field experiment with a randomized complete block design. Treatment factors included two main factors of global change, warming and nitrogen addition (two levels each), in addition to three levels of silicon supplied to plants during growth. The simulated global-change factors applied to enclosures at the climate-change facility resulted in four treatments: controls (C) where water was kept at ambient temperature, warming (H) where water temperature was raised above ambient by active heating (average of 2.03°C during the study period), enhanced nitrogen supply (N) achieved by monthly addition of dissolved Ca(NO₃)₂ to reach target concentrations about 5× above the ambient concentration, and warming and enhanced nitrogen supply in combination (HN; Appendix: Figs. A1 and A2). The targeted fivefold increase in N concentrations is consistent with increased nitrogen loads observed in northern temperate lakes (Elser et al. 2009) and the

pulsed monthly additions simulated nitrogen delivery occurring during rain events (Hines et al. 2013). An unenclosed control plot (L) was also established to test for potential enclosure effects. Thus, five treatments were replicated in each of four random blocks, resulting in a total of 20 experimental units.

Each treatment was randomly assigned to an enclosure in the field facility that had been running continuously with minor interruptions since 2004, as described in detail by Hines et al. (2013). Briefly, each enclosure consisted of two nested polypropylene rings with an inner and outer diameter of 1.42 and 1.52 m, respectively. Enclosures were pushed 20 cm into the sediment, resulting in a height of 1.2 m (Hines et al. 2013). They were open at the top and bottom and enclosed naturally grown shoots of *P. australis*. The total enclosure volume depended on the lake water level but was about 1 m³ at a water depth of 65 cm. The silicon concentration in the lake and enclosure water ranged between 1 and 9 mg/L during the experiment, but did not differ among treatments.

Litter containing three different levels of silicon were obtained from a separate experiment where *P. australis* was grown in polyethylene pots (15 L) filled with purified water and 1 kg of peat containing 10% Si by dry mass. The plants were exposed to silica treatments in a pot experiment that was separate from the global change enclosures. The pots with 10–25 *Phragmites* shoots each were placed in open-top chambers on land and supplied with filtered air. Twelve pots per treatment received one of three levels of low acidic (pH 4.7), amorphous Si (Aerosil 300; Evonik Industries AG, Essen, Germany): 0 g (Si-C), 10 g (Si-10), and 100 g silica (Si-100). Further details on growth conditions are given in Schaller et al. (2012b). Litter was harvested at the end of the growing season (19 November 2009) when shoots had turned brown. They were separated into leaf blades, leaf sheaths, and culms. Litter chemistry differed among the plant organs and Si treatments in terms of Si, N, P, and slightly for cellulose and phenol content (Schaller et al. 2012a, b).

Litter chemistry and mass loss

The harvested fully brown leaf blades, sheaths, and culms were placed in separate litter bags (20 × 25 cm) with a mesh size of 1 mm. Each bag received 0.8–1.1 g of litter ash free dry mass (AFDM). There was insufficient sheath material to include a treatment in the unenclosed control plots (L), resulting in a total of 180 litter bags that were submerged in the enclosure facility in Lake Hallwil in early December 2009. Litter bags were retrieved after about 50 % of the initial mass had been lost (checked by periodically sampling extra leaf bags); therefore, bags containing leaf blades and sheaths were retrieved in mid April 2010 after four months, those containing culms in December 2010 after exactly one year.

The litter was cleaned under flowing water, frozen at –20°C, and later freeze dried, ground to a particle size <0.5 mm using a centrifugal mill (ZM 1000; Retsch, Hanau, Germany), and the leaf blades analyzed for carbon, nitrogen, phosphorus, and silicon using the same analytical methods described previously for undecomposed litter (Schaller et al. 2012a, b). Briefly, carbon and nitrogen contents were determined using an Elementar Vario EL III (Retsch) elemental analyzer (Deutsches Institut für Normung 1995). Litter for phosphorus analysis was digested in 3 mL of HNO₃[–] and 2 mL of H₂O₂ (Deutsches Institut für Normung 2002). For silicon analysis, ground litter was digested in a microwave digestion system (Mars5; CEM Corporation, Matthews, North Carolina, USA) in 3 mL of HNO₃, 1.5 mL of HF, and 3 mL of H₃BO₃ (DIN-EN-13 805 2002). Standard reference material (poplar leaves, GBW7604; Office of Certified Reference Material, Langfang, China) was processed along with the samples. Silicon and phosphorus were determined by ICP-OES (Optima 7000DV; Perkin Elmer, Waltham, Massachusetts, USA) with UV detection and quantification at 251.6 nm (Si) and 213.6 nm (P). All chemicals were of analytical grade. To further examine the extent of silica deposition in plant tissue, leaf blades from plants grown under high Si supply (Si-100) were examined by scanning electron microscopy to determine carbon and silicon concentrations near the epidermis. Leaves of both undecomposed material and samples retrieved from the field were inspected. The microscope (JEOL T 330A; Röntec, Berlin, Germany) was equipped with an element detector (EDR 288; Röntec) and run at 15 kV. Plant samples were critical-point dried and subsequently coated with gold (Zimmermann et al. 2000). An area of about 0.09 mm² in the middle of selected leaf blades was scanned at a magnification of 350×.

Fungal biomass

Ergosterol in leaf blades was determined as a measure of fungal biomass. Freeze-dried and ground leaf material (50 mg) was extracted with 10 mL of KOH/methanol (8 g/L KOH) for 30 minutes at 80°C (Gessner and Schmitt 1996). Extracts were acidified with 2 mL of 0.65 mol/L HCl and loaded on solid-phase extraction cartridges (tC18; Waters No. WAT 043425; Waters Corporation, Milford, Massachusetts, USA). Cartridges were rinsed with a solution containing 22.4 g KOH per liter of methanol/water (6:4 by volume) and dried for about an hour under a stream of air. Ergosterol was eluted with 1750 µL of isopropanol (Gessner and Schmitt 1996). The resulting extracts were analyzed for ergosterol on a Jasco HPLC (Jasco, Gross-Umstadt, Germany) with methanol as the mobile phase (flow rate of 1.5 mL min^{–1}) using a 250 × 4 mm LichroSpher RP18 column (Merck No. 327 799) maintained at 33°C. Ergosterol was detected and quantified by measuring absorbance at 282 nm.

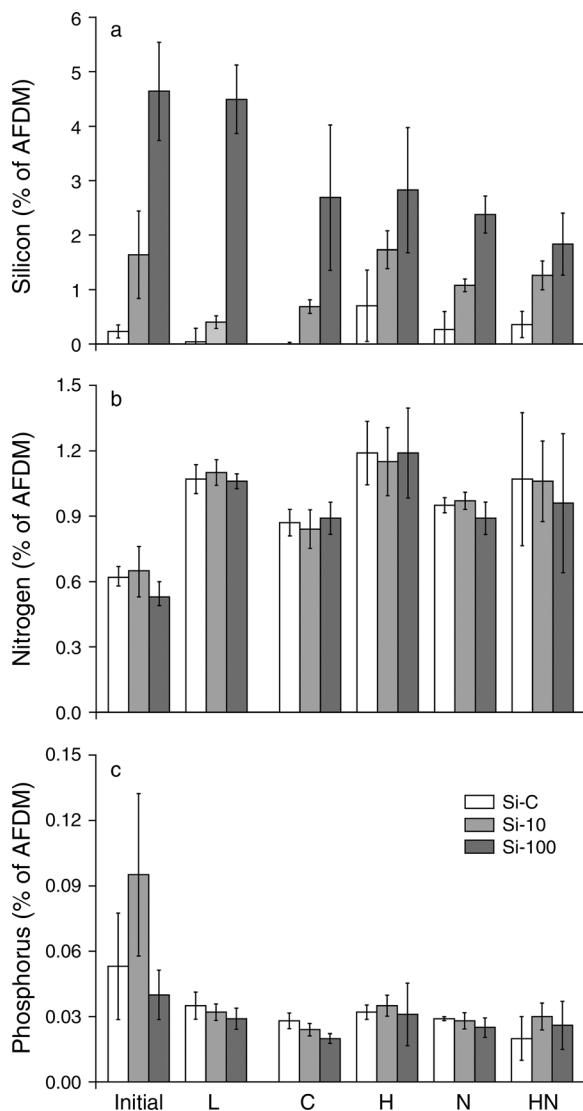


FIG. 1. (a) Silicon, (b) nitrogen, and (c) phosphorus concentrations (means \pm SD, $n = 4$ replicates) of *Phragmites australis* leaf blades at the beginning and end of a litter decomposition experiment in different environmental conditions: L, unenclosed control plot in a littoral marsh; C, untreated control enclosures; H, heated enclosures; N, enclosures receiving NO_3^- additions; and HN, heated enclosures receiving NO_3^- additions. Initial denotes undecomposed litter, all other labels on the x-axis refers to litter retrieved in early April after four months of decomposition. Treatments are Si-C, no silicon fertilization; Si-10, 10 g silicon added per 10 L of substrate during plant growth; and Si-100, 100 g silicon added per 10 L of substrate. AFDM stands for ash-free dry mass.

Statistical analysis

Separate three-way analyses of variance (ANOVA) were used to test for treatment effects on litter mass loss of leaf blades, leaf sheaths, and culms of *P. australis*. The factors included were warming (two levels), nitrogen addition (two levels), and silica during plant growth (three levels). The block factor was not significant and

was therefore removed from the analysis. For leaf blades, we also assessed treatment effects on changes in litter chemistry occurring during decomposition (carbon, nitrogen, phosphorus and silicon concentrations), and fungal biomass measured as ergosterol content. To assess the potential for enclosures to affect results, we compared decomposition of all plant tissues in enclosed and unenclosed controls using two-way ANOVA with the presence of enclosures and Si availability during plant growth as categorical factors. All data met assumptions of homogeneity of variance and normal distribution of residuals. All statistical computations were performed with SPSS version 14.0 (IBM, Armonk, New York, USA).

RESULTS

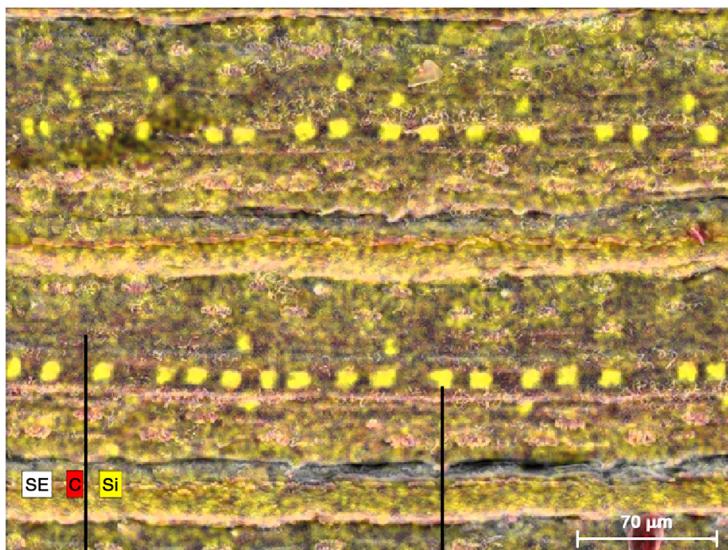
Litter chemistry

The initial concentrations of silicon in leaf blades differed 20-fold among plants supplied with varying amounts of Si during growth (Fig. 1a). Amorphous silica deposits initially present on the leaf surfaces of the plants receiving the highest Si concentrations had disappeared by the time of sampling after four months, and some of the silica bodies (phytoliths) appeared to have been partly dissolved by this time (Fig. 2). Nevertheless, differences in Si content among litter from plants grown under different Si supply regimes persisted in all cases (Fig. 1a), but did not influence other aspects of litter chemistry, either directly or interactively with other factors of global change (Fig. 1b, c, Appendix: Table A1). Nitrogen concentration in leaf litter consistently increased during decomposition, in some cases nearly doubling (Fig. 1b), whereas phosphorus concentrations decreased (Fig. 1c). These changes occurred across all treatments, independent of Si supply during plant growth. Warming and external nitrogen in combination enhanced the litter phosphorus concentration compared to the control litter (Table A1). The nitrogen concentration was not affected. However, warming enhanced the final concentrations of nitrogen in leaf litter, whereas external nitrogen supply had no influence (Fig. 1b, Table A1). Similarly, warming enhanced the final concentration of phosphorus, but only when no extra nitrogen was supplied (Fig. 1c), resulting in a significant interaction effect of warming and nitrogen supply on phosphorus (Table A1). Initial differences in the carbon content of plants exposed to different Si supply levels tended to diminish during decomposition, but were still apparent after four months, except in leaf litter retrieved from the heated enclosures (Fig. A3, Table A1).

Fungal biomass

The clearest and most consistent responses to altered Si supply and environmental conditions emerged for ergosterol, a measure of fungal biomass (Fig. 3). High silicon supplies during plant growth reduced ergosterol concentrations in leaf litter by about half compared to concentrations in litter from plants exposed to low and medium levels of Si ($P = 0.014$; Table A1). This effect was

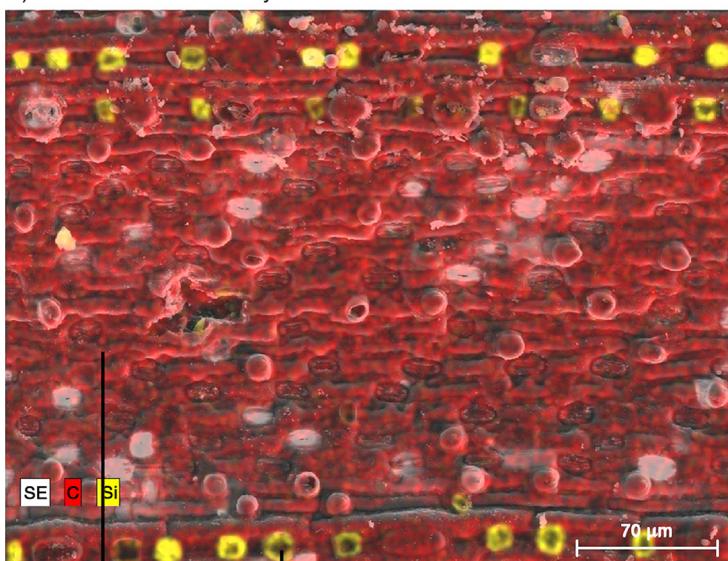
a) Leaf surface before decay



Amorphous silica deposits

Distinctly arranged silica bodies

b) Leaf surface after decay



Partly dissolved silica body

Site of dislodged amorphous silica deposits

FIG. 2. False-color scanning electron microscopy (SE) images showing the distribution of silica bodies and copious amorphous silica deposits in and on the uppermost surface layer of leaf blades of *P. australis* plants receiving a high dose of Si (46.6 g per 10-L bucket) during plant growth (Si-100) (a) before and (b) after four months of litter decomposition (early December to early April) in unenclosed control plots in a freshwater marsh. Silica appears in yellow, carbon in red. Magnification 350 \times .

consistent across all treatments in enclosures, including the unenclosed control plots. Furthermore, ergosterol in leaf litter was strongly affected ($P < 0.001$) by N addition during decomposition (Fig. 3), with concentrations consistently lowered across all Si treatments, again by about 50%. Warming, in contrast, had no effect on ergosterol concentration, nor were there significant interactive effects between Si supply of plants, nitrogen

enrichment and warming (Table A1). The highest ergosterol concentrations were observed in the unenclosed control plot, indicating a negative enclosure effect on ergosterol concentration in the decomposing litter.

Litter mass loss

The influence of global-change factors on litter mass loss varied depending upon the plant tissue considered:

leaf blades and sheaths responded similarly, whereas mass loss of culms followed a different response pattern (Fig. 4). Specifically, experimental warming increased the mass loss of *P. australis* litter for leaf blades ($P = 0.001$) and sheaths ($P = 0.023$), in spite of notable variability among replicates for most of the warming treatments (Fig. 4a, b). In contrast, N addition had no significant effects on the mass loss of either tissue (Fig. 4a, b). Furthermore, elevated Si supply during plant growth resulted in consistently faster decomposition of leaf sheaths ($P = 0.017$), although for leaf blades the same effect was only observed within the lake control treatment ($P = 0.016$ for the Si effect in unenclosed plots vs. $P = 0.41$ in enclosures). Culm litter decomposition, in contrast to that of leaf blades and sheaths, was not influenced by either warming ($P = 0.19$) or Si supply during plant growth ($P = 0.69$), but instead was affected by N addition ($P < 0.001$), which slowed the mass loss of culms by about 25% (Fig. 4c). No interactive effects on litter mass loss were observed for any of the three plant tissues (Table A2). Enclosures had a negative effect on the decomposition of leaf blades ($P < 0.001$) and culms ($P < 0.005$), resulting in slower mass loss in the enclosed control plots compared to the unenclosed controls (Fig. 4a, c).

DISCUSSION

Environmental heterogeneity caused by variation in Si availability clearly affected litter chemistry (C and P concentration) and fungal decomposers, whereas the effects of warming and N supply on litter decomposition proved to be more complex. In particular, increased Si in leaf blades approximately halved fungal biomass accrual during decomposition, but this Si effect did not correspond with the patterns observed for litter mass loss. The resulting uncoupling of fungal growth and decomposition indicates that microbes other than fungi assumed an important role in the decomposition process. Bacteria are prime candidates as alternative decomposers, especially in view of their high productivity on submerged litter (Buesing and Gessner 2006) and the gradual declines of fungal biomass when decomposition of submerged litter proceeds in marshes (Kuehn et al. 2000, Van Ryckegem et al. 2007). This suggests that silica may influence decomposition by altering the composition and relative importance of particular groups of microbial decomposers.

The influence of Si on decomposition depended upon litter tissue type. Specifically, only sheath decomposition was accelerated (Fig. 4). This has also been observed in another study (Schaller and Struyf 2013) and might be due to the particularly high accumulation of Si in sheath tissue (Schaller et al. 2013). However, the stimulation of sheath decomposition is at variance with the common interpretation that elevated Si concentrations in plants act as a defense mechanism (Fauteux et al. 2005, Massey and Hartley 2009), which should remain functional after plant death (Cornelissen et al. 2004) and hence reduce

microbial decomposer activity. A possible explanation for this apparent paradox is that high Si concentrations shift the outcome of competitive interactions between fungi and bacteria (Mille-Lindblom and Tranvik 2003) in favor of the latter (Wainwright et al. 1997, Tian et al. 2012). Such a shift could be facilitated by an increase in the surface area of litter available for bacterial colonization when phytoliths and amorphous silica deposits are partially solubilized or mechanically removed during decomposition (Fig. 2; also see Holstein and Hensen 2010). Such a surface area effect could also explain the lacking influence of Si on the mass loss of leaf blades and culms, because silicon contents and condensation state of these litter types are lower than in sheaths (Schaller et al. 2013). Importantly, fungi are unlikely to benefit in similar ways from surface-area enlargements, because their hyphae penetrate the litter surface to expand their mycelial network within the decomposing plant tissue (Newell et al. 1996).

Temperature increases affected the three litter types (leaf blades, sheaths, and culms) differentially, suggesting that differences in plant tissue chemistry of *P. australis* not only influence decomposition in current conditions, but also determine sensitivity to climate warming. Leaf-blade decomposition, which was the fastest of the three tissues, was accelerated by warming. Leaf sheaths, which on average lost 10% less mass than leaf blades, responded less strongly to warming but instead lost mass significantly faster at elevated Si supply levels (see above). This contrasts again with culm litter, which responded neither to warming nor to litter Si content. Thus, tissue specific differences in litter chemistry as well as plasticity in the ability to accumulate Si clearly influence decomposition responses to changing environmental conditions.

That temperature effects on litter mass loss arose only for leaf blades and sheaths could in part be due to the much faster decomposition of these tissues compared to that of culm litter, resulting in 40–50% mass loss of leaf blades within four months at the low winter temperatures. Increased N immobilization in this condition (Fig. 1b), which suggests enhanced microbial activity, further supports this interpretation. The additional heat input applied by our warming treatment during winter was much more important, in relative terms, than the experimental extra warming experienced by culm litter exposed to the full seasonal cycle (12 months) during which temperatures exceeded 20°C for many months. This suggests that climate warming effects could be more pronounced during winter (Easterling et al. 2000, Raisanen et al. 2004). That is, the divergence in the response of different litter types to warming may only emerge when litter decomposes rapidly enough to achieve substantial mass loss during the cold winter months. Although this interpretation only holds if decomposer activity declines, or at least slows, at the elevated temperatures prevailing in summer, evidence from microbial decomposers in streams (Suberkropp

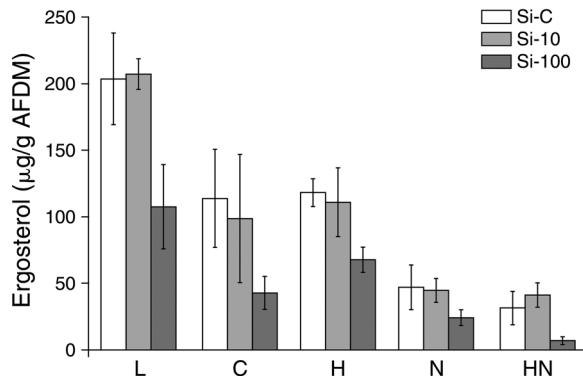


FIG. 3. Ergosterol concentrations (means \pm SD, $n = 4$ replicates) in *P. australis* leaf blades after four months of decomposition (early December to early April) in different environmental conditions. Treatments and abbreviations are as in Fig. 1.

1984, Dang et al. 2009) suggests that this is not an unrealistic assumption.

Similar to effects of Si, increased external N supply had a strong influence on fungi that did not directly correspond to changes in litter mass loss. Indeed, fungal biomass in leaf blades subjected to high Si and N in combination was consistently reduced to only about 25% of the fungal biomass found on leaf blades in control enclosures, a perfect additive effect on a relative scale. This finding that synergistic or antagonistic effects of multiple environmental drivers do not necessarily complicate projected impacts of microbial decomposers on plant litter is encouraging. It suggests that realistic large-scale models of future litter decomposition and related processes remain within reach. However, mixed responses among tissue types and process rates demonstrate that increased attention needs to be directed to the impacts of environmental heterogeneity on plant tissue quality as a key factor affecting variation in decomposition and associated processes.

As with other effects, the mixed responses of decomposition to N supply shown by leaf blades and sheaths (i.e., no response) vs. culm litter (negative response) could be explained by differences in litter quality. In forest soils, recalcitrant or partly degraded litter is often inhibited by experimental N supply, contrasting with stimulated decomposition rates of fresh litter that is relatively labile (Hobbie et al. 2012). Accordingly, N supply significantly slowed the decomposition of recalcitrant culm litter in our experiment, whereas that of rapidly decomposing leaf blades and sheaths (Gessner 2000) was unaffected. Thus, nitrogen effects were prominent on the tissue that decomposed the slowest, contrasting with warming effects that were most pronounced on the fastest decomposing tissue. The mechanism underlying the divergence between litter types in their response to external N supply remains uncertain, but because bacteria often become more important as litter becomes more refractory with

decomposition progressing (Gessner et al. 2007), it could be that N addition limited bacterial activity. Evidence from forests suggests that N can inhibit fungal oxidative enzymes (peroxidases) essential for the degradation of lignin, a highly refractory compound that accounts for a large fraction of recalcitrant litter (Carreiro et al. 2000). Although effective lignin-degrading fungi are less common in fresh waters than in forests (Gulis et al. 2006), the same mechanism could work for ligninolytic enzymes of aquatic bacteria (Vicuna 1988, Bugg et al. 2011), and it would also be in line with the 50% decline in fungal

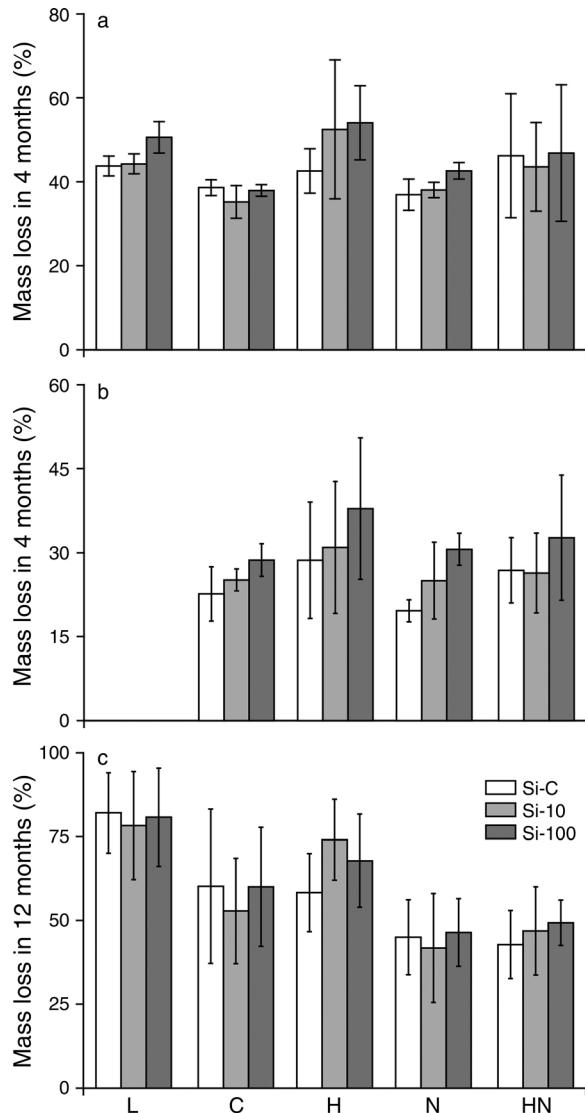


FIG. 4. Loss in ash-free dry mass (AFDM; means \pm SD, $n = 4$ replicates) of (a) leaf blade, (b) leaf sheath, and (c) culm litter of *Phragmites australis*. Plants had been grown at three different silicon supply levels and decomposed in submerged litter bags for 4 months (129 days, from early December to early April, for leaf blades and sheaths) or 12 months (365 days, from early December to early December, for culms). Treatments and abbreviations are as in Fig. 1.

biomass that we observed when excess N was supplied to decomposing leaf blades (Fig. 3).

In conclusion, important outcomes of our experiment are that (1) Si availability during plant growth strongly impacts fungal decomposers, (2) high external N supply produces equally strong negative effects on fungi, but (3) these negative impacts of Si and N on fungi do not invariably translate to slowed decomposition. This could suggest that bacteria in freshwater marshes play a greater role in the decomposition of submerged litter than is commonly recognized. In addition, (4) differences in plant tissue quality within individual species, exaggerated by environmental heterogeneity such as Si availability, can influence impacts of global change on decomposition. These effects could be particularly pronounced in light of human alteration in the biological availability of multiple chemical elements such as nitrogen (Galloway et al. 2008) and silicon (Struyf and Conley 2009, Vandevenne et al. 2012).

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SUPPLEMENTAL MATERIAL

Ecological Archives

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