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Genotypic trait variation modifies effects of climate warming and nitrogen deposition on litter mass loss and microbial respiration

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Abstract

Intraspecific variation in genotypically determined traits can influence ecosystem processes. Therefore, the impact of climate change on ecosystems may depend, in part, on the distribution of plant genotypes. Here we experimentally assess effects of climate warming and excess nitrogen supply on litter decomposition using 12 genotypes of a cosmopolitan foundation species collected across a 2100 km latitudinal gradient and grown in a common garden. Genotypically determined litter-chemistry traits varied substantially within and among geographic regions, which strongly affected decomposition and the magnitude of warming effects, as warming accelerated litter mass loss of high-nutrient, but not low-nutrient, genotypes. Although increased nitrogen supply alone had no effect on decomposition, it strongly accelerated litter mass loss of all genotypes when combined with warming. Rates of microbial respiration associated with the leaf litter showed nearly identical responses as litter mass loss. These results highlight the importance of interactive effects of environmental factors and suggest that loss or gain of genetic variation associated with key phenotypic traits can buffer, or exacerbate, the impact of global change on ecosystem process rates in the future.

Keywords: global change, interactive global-change effects, intraspecific genotypic diversity, litter decomposition, *Phragmites australis*

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Introduction

Projected increases in global temperatures by 1.5–4.5 °C in the coming decades have spurred interest in the consequences of warming for ecosystem dynamics (Davidson & Janssens, 2006; Solomon *et al*., 2007). Temperature increases usually accelerate biological activity (Gillooly *et al*., 2001; Dell *et al*., 2011), but whether the strong temperature sensitivity of metabolic rates will also translate into ecosystem-level effects of climate warming is debated (Davidson & Janssens, 2006; Walther, 2010). This is, in part, because real ecosystems are simultaneously confronted with multiple factors of environmental change, which can directly alter biogeochemical processes and also select for changes in species traits (Walther *et al*., 2002; Parmesan *et al*., 2013). Predicting the consequences of these combined effects can be challenging (Vinebrooke *et al*., 2004; Ormerod *et al*., 2010). For example, warming typically accelerates the decomposition of plant litter (Fierer *et al*., 2005; Dang *et al*., 2009; Ferreira & Chauvet, 2011), one of the most important ecosystem processes in the biosphere, but the stimulation could be offset by other factors of global environmental change such as excessive nitrogen supply. Burning of fossil fuels and increased use of agricultural fertilizers have greatly increased inputs of reactive nitrogen into both terrestrial and aquatic ecosystems worldwide (Galloway *et al*., 2008; Deegan *et al*., 2012), and the consequences on ecosystems have received much attention (Hyvonen *et al*., 2007; Flury & Gessner, 2014). However, considerable uncertainty exists about the consequences of external nitrogen supply for decomposition (Bragazza *et al*., 2006; Hobbie, 2008). On one hand, there is evidence suggesting that nitrogen supply delays decomposition by inhibiting fungal lignin-degrading enzymes (Carreiro *et al*., 2000). On the other hand, nitrogen has been repeatedly observed to accelerate decomposition (Bragazza *et al*., 2006; Hobbie, 2008). Thus, it currently remains an open question whether external nitrogen will accelerate or delay litter decomposition in a warmer world.

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One of the main factors that determines current rates of litter decomposition, as well as the potential sensitivity of leaf litter decomposition to global environmental change, is the chemical composition of the litter itself (Knorr *et al*., 2005; Ferreira & Chauvet, 2011). Particular attention has been given to nitrogen (N) and phosphorus (P) as essential and often limiting nutrients for microbial decomposers (Enriquez *et al*., 1993), as well as to carbon compounds such as lignin (Wieder *et al*., 2009). Often highly correlated, the specific role of each chemical component has been difficult to tease apart (Talbot & Treseder, 2012). Nonetheless, it is clear that initial litter chemistry, whether determined by a single factor or a combination, influences rates of litter mass loss and associated processes in a wide variety of ecosystems (Melillo *et al*., 1982; Gessner & Chauvet, 1994; Wickings *et al*., 2012).

Traditionally, much of the variation in leaf chemistry has been attributed to varying environmental conditions (Reich & Oleksyn, 2004; Wright *et al*., 2004; Santiago, 2007) or species-specific differences (Cornwell *et al*., 2008). However, a recent body of research has shown that genetic variation *within* species can also notably alter expression of key phenotypic traits such as litter chemistry (Madritch & Hunter, 2002; Schweitzer *et al*., 2004). Such intraspecific differences can have strong impacts on the structure and function of biological communities (Crutsinger *et al*., 2006; Hughes *et al*., 2008; Bailey *et al*., 2009), which suggests that genotypic variation in litter chemistry could play a key role in determining ecosystem sensitivity to global environmental changes, including climate warming and nitrogen supply.

Notably, information about the current distribution of genotypic variation is scarce for many important plant species (McMahon *et al*., 2011; Bailey *et al*., 2013), and current distributions are likely to shift if global change affects the establishment or survival of genetic variation association with particular traits (Davis & Shaw, 2001; Avolio *et al*., 2013). Such range expansions and contractions in response to global change can be easily observed when the species composition of plant communities is altered (Walther *et al*., 2002), but they typically remain cryptic when they transpire as genotypic range shifts of currently established, broadly distributed species (Davis & Shaw, 2001; Saltonstall, 2002). Will loss or gain of intraspecific variation in such broadly distributed species influence the impact of global environmental change on important ecosystem processes such as plant litter decomposition?

We assessed how genotypic variation in the litter chemistry of a cosmopolitan foundation species, *Phragmites australis,* influences rates of litter mass loss and microbial respiration in current and near-future global-change scenarios. Toward this goal, we used an outdoor experimental facility designed to simulate climate warming and increased nitrogen deposition in factorial combination in realistic field conditions (Hines *et al*., 2013). Use of twelve distinct clones originating from three regions across a broad latitudinal gradient and grown in a common garden allowed us to determine the extent to which variation in current climate conditions has selected for genotypes with particular traits. Specifically, we hypothesized that (i) genotypic differentiation across broad latitudinal gradients would be the primary driver of variation in litter traits important for decomposition, while trait variation of genotypes originally collected from similar environments within the same geographic regions would be relatively small; (ii) warming would accelerate litter mass loss and microbial respiration, and the effect would be stronger for high-nutrient compared with low-nutrient genotypes; (iii) nitrogen addition would accelerate mass loss and microbial respiration of high-nutrient genotypes, but have the opposite effect on low-nutrient genotypes (Hyvonen *et al*., 2007); and (iv) when acting simultaneously, genotypic variation, warming, and excess nitrogen supply would produce additive effects on mass loss and respiration. Taken together, these hypotheses address complex genotype by environment interactions (GxExE) with the overall goal of revealing mechanisms behind variation in the potential impacts of global environmental change on ecosystem processes that would remain unnoticed if analyses are limited to variation among traits at the species level only.

Materials and methods

Study system

We chose wetland ecosystems composed of *Phragmites australis* (Cav.) Trin. ex. Steud. to assess the combined effects of plant genotypic variation and two factors of global environmental change (climate warming and nitrogen loading) on plant litter mass loss and microbial respiration. *P. australis* is one of the most broadly distributed angiosperms worldwide (Mal & Narine, 2004). As a foundation species, it commonly forms large monospecific stands that are highly productive (Gessner *et al*., 1996), so that incomplete decomposition can result in substantial carbon sequestration through peat accumulation (Brix *et al*., 2001). Highly competitive and hence precariously invasive in parts of the world (North America), *Phragmites* is of high conservation value on other continents where it previously underwent strong declines (Europe). Importantly, the species is clonal and shows substantial genetic variability (Saltonstall, 2011). Given the importance of wetlands for the global carbon cycle (Mcleod *et al*., 2011; Bridgham *et al*., 2013) and the widespread occurrence and clone-specific differences in *Phragmites* (Hansen *et al*., 2007), this plant provides an excellent model to examine the combined impact of genotypic variation and global-change factors on ecosystem processes.

Litter material

Rhizomes of *P. australis* were collected from four sites in each of three regions spanning a 2100 km latitudinal gradient along the North Atlantic coast of North America. The 12 clones were grown in a common garden in an unshaded field at the Smithsonian Environmental Research Station in Edgewater, MD, USA (38°57′25″N/76°32′59″W). Restriction-fragment length polymorphism (RFLP) analysis indicated that distinct clones were collected from four different locations within each of the three geographic regions (Northeast, Mid-Atlantic, Southeast United States), and that the Southeast clones were subspecies *P. australis berlandieri*, whereas both the Northeast and Mid-Atlantic clones were subspecies *P. australis australis*, which is representative of the spatial distribution of this species (Saltonstall, 2002). Three replicate plants of each clone were propagated by placing preweighed rhizomes in soil-filled plastic pots (60 cm diameter, 46 cm depth filled with Metro-mix 510 (R) soil to a depth of 40 cm). The pots were positioned in a plastic wading pool (130 cm diameter, 30 cm depth) that was permanently flooded to 20 cm depth with fresh water. Following senescence, the aboveground biomass was harvested and air-dried to constant mass. Leaves from the three replicate pots for each clone were pooled, and gently tumbled by hand to homogenize the litter. Subsamples of the litter were ground in a modified planetary ball mill (PM 400, Retsch GmbH, Haan, Germany). The initial cellulose and lignin contents of the litter were determined gravimetrically after sequential removal of cell constituents using a modified acid-detergent fiber method described in detail by Gessner (2005) and Flury & Gessner (2014). Acid-detergent fiber was extracted from 200 mg of ground plant material by boiling in 0.5 M sulphuric acid amended with 20 g l^{-1} hexadecyltrimethylammonium bromide (CTAB). The remaining material was collected, rinsed, dried, and weighed on a Gooch crucible. Cellulose was subsequently hydrolyzed with 72% H_2SO_4, and lignin was determined in the residue as mass loss during ignition in a muffle furnace. Total nitrogen and phosphorus content of the leaf litter was determined using standard spectrophotometric assays after digestion of ground litter material (Ebina *et al.*, 1983; Flury & Gessner, 2014).

Global change field experiment

A decomposition experiment was conducted in an enclosure facility located within a natural *Phragmites* marsh on the shore of Lake Hallwil, Switzerland (47°17′N, 8°14′E) (Hines *et al.*, 2013). The enclosures were designed to simulate climate warming (ambient vs. ambient +2.8 °C) and increased nitrogen supply (ambient vs. 5× ambient) using a 2 × 2 factorial design. A fifth unenclosed control treatment was included to assess effects of the enclosures. Each of the five treatments was assigned a random location in each of four spatial blocks (16 enclosed plots + 4 unenclosed plots = 20 experimental units in total) (Hines *et al.*, 2013).

The design, functioning, and maintenance of the enclosures, which have been running with minor interruptions since 2004, are described in detail elsewhere (Hines *et al.*, 2013). Briefly, each enclosure consisted of two nested polypropylene cylinders (1.4 m and 1.5 m diameter) pushed 20 cm into the sediment. The warming treatment was achieved by active heating with electric heaters designed for koi ponds. To assure that the warming treatment paralleled the natural diel, daily and seasonal temperature fluctuations in the marsh, programmable temperature controllers switched the heaters off and on when the temperature in the heated enclosures exceeded or fell below the target temperature. Increased nitrogen supply was simulated by adding a total of 22.5 g N per year (=14.2 g N m^{-2} yr^{-1}), which was applied monthly as calcium nitrate [$Ca(NO_3)_2 \times 4H_2O$] dissolved in lake water. Long-term monitoring of ambient surface waters suggests that our calcium additions made marginal contributions to the naturally hard water of Lake Hallwil, effectively minimizing changes in water chemistry other than nitrate (Hines *et al.*, 2013). The N concentrations for these monthly nitrate additions paralleled natural fluctuations in dissolved N concentrations of the lake. They were designed to simulate nitrate pulses experienced during and after precipitation: January 1.9, February 1.9, March 2.6, April 2.6 g N m^{-2} (Hines *et al.*, 2013).

To determine rates of litter mass loss and litter-associated microbial respiration, fine-mesh bags (280 μm nylon mesh) were filled with 1.0 ± 0.1 g of leaf litter from each of the 12 genotypes, deployed in each enclosed and unenclosed plot on 26 January 2011, and retrieved again after 54 days (21 March 2011) and 82 days (18 April 2011). Thus, a total of 480 fine-mesh litter bags were used in the experiment (12 genotypes × 20 field plots × 2 sample dates). Litter bags collected from the marsh on both sampling dates were transported to the laboratory where leaves were gently washed to remove silt. Measurements of litter mass loss provide an estimate of multiple processes contributing simultaneously to decomposition (Cotrufo *et al.*, 2010). Therefore, to provide a second independent and more specific estimate of microbial decomposition, we also placed leaves in sealed respiration chambers and determined respiration rates using fiber-optic sensors (Fibox Oxy10; Presens GmbH, Regensburg, Germany). Respiration chambers were filled with filtered lake water (0.45 μm cellulose acetate membranes) maintained at temperatures consistent with seasonal lake temperatures (6 °C on 21 March 2011, and 8 °C on 18 April 2011). Water in the respiration chambers was constantly stirred during measurements using motorized stirring paddles. Subsequently, all leaf material was oven-dried for 48 h at 50 °C and weighed to the nearest 0.01 g.

Statistical analysis

To assess variation and covariation in initial litter chemistry among genotypes, we conducted a principal components analysis using standardized data (*z*-scores) of nitrogen, phosphorus, lignin, and cellulose content. We also examined all possible relationships among initial litter-chemistry traits using Pearson's correlation coefficient (*r*). Mass loss rate

coefficients (k) were calculated using an exponential decay model ($M_t = M_o^{-kt}$), where M_t = mass at time t, and t = elapsed time in days. Subsequently, we used a mixed model ANCOVA (PROC MIXED, SAS 2001) to examine the influence of global-change factors on the relationship between initial litter chemistry and rates of litter mass loss and respiration. Because litter-chemistry traits covaried strongly, we used Akaike's Information Criterion (AIC) values to choose the litter-chemistry response covariate that best fits the data. Initial P content of the 12 clones was the genotypic trait yielding the lowest AIC, but initial N content produced nearly identical results. The five global-change treatments (warming, nitrogen loading, warming + nitrogen loading, enclosed control, open-marsh control) were considered as fixed categorical factors. Following significant results for the overall ANCOVA, we used the fully crossed factorial treatment structure to examine the direct and interactive effects of the two global-change factors, warming and nitrogen loading. Additionally, we used *a priori* contrasts to compare decomposition and respiration in the enclosed vs. the unenclosed control. Random block factors included enclosure and spatial block.

Results

Growth and senescence of the 12 plant genotypes from distinct locations but grown in a common garden resulted in strong intraspecific variation in litter chemistry (Fig. 1), demonstrating that, in the same environmental conditions, genotypes differentially expressed phenotypic traits fundamental for litter decomposition. Three striking patterns in litter chemistry emerge. First, there was three- and sevenfold variation, respectively, in initial litter nitrogen (0.6–1.8%) and phosphorus (0.2–1.4%) content among the 12 clones grown in identical environmental conditions (Fig. 1). Second, there was strong covariation among litter chemistry variables (Fig. 1), particularly between litter N and P contents, which showed a very tight positive correlation ($r = 0.98$, $P < 0.0001$). Concentrations of both elements were also negatively correlated with litter lignin content ($r_{\text{N-lignin}} = 0.61$, $P = 0.03$; $r_{\text{P-lignin}} = 0.58$, $P = 0.05$; Fig. 1), indicating consistent

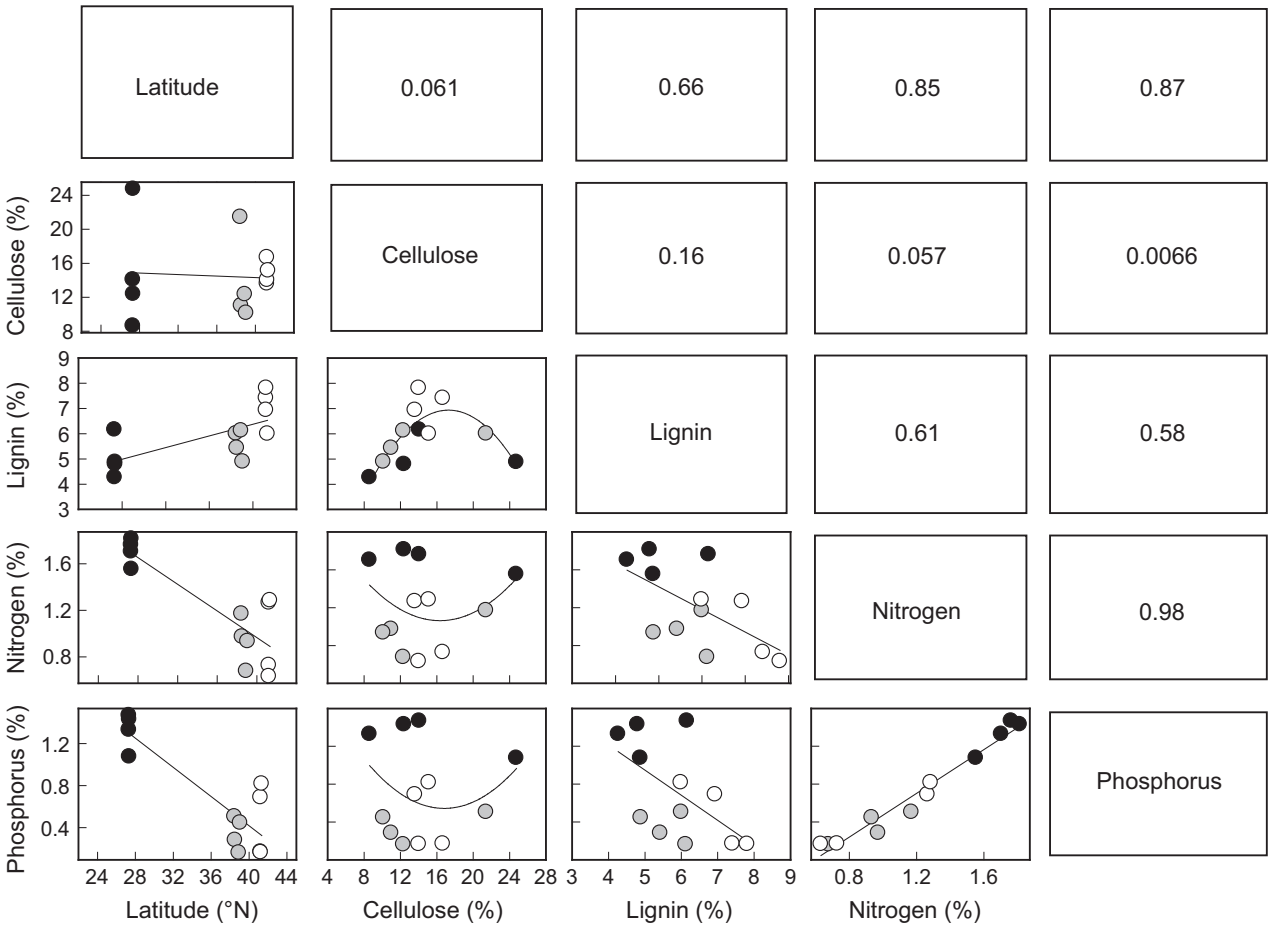

Fig. 1 Intraspecific variation in litter-chemistry traits of *Phragmites australis* clones grown in a common garden. Panels below the diagonal show covariation of litter traits (% dry mass of cellulose, lignin, nitrogen, and phosphorus) and smoothed lines show locally weighted regression (Clevland & Devlin, 1988). Panels above the diagonal show Pearson's correlation statistic (r) of traits in the associated column and row. In all panels gray tones indicate region of origin for each genotype (latitude °N): black – low, gray – mid, and white – high latitude.

variation across a range of key chemical constituents defining litter quality for decomposers. Third, there was substantial variation in litter chemistry both across and within latitudes. The genotypes collected from four different low-latitude locations consistently expressed the highest nutrient (N and P) concentrations and thus strongly influenced the overall nutrient gradient across clones. However, nutrient contents also varied twofold (0.6–1.2% N) or even fourfold (0.2–0.8% P) among the eight clones originating from mid- and high-latitudes (Fig. 1).

Overall, litter mass loss of our 60 individual treatments (12 genotypes × 5 environmental conditions) conformed well to an exponential decay model, although regression lines were based on two sampling occasions only (Fig. 2). On average, litter retrieved from the field had 63% mass remaining on day 54% and 51% remaining on day 82 (Fig. 2). However, the rate of litter mass loss varied notably across genotypes and global-change treatments (Figs. 2 and 3; Table 1). The best predictor of clone-specific differences in litter mass loss rate was litter phosphorus content (Table 1). It

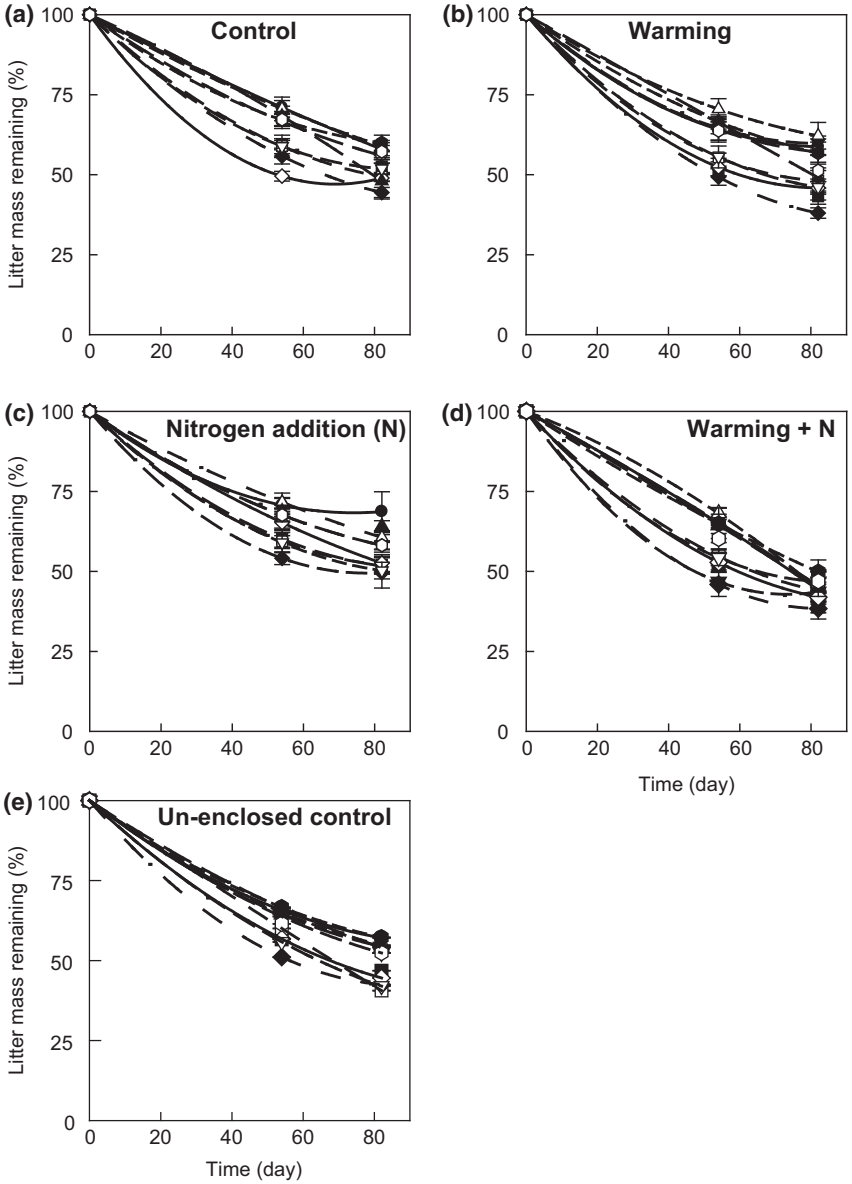

Fig. 2 Percent mass loss of leaf litter from 12 *Phragmites australis* clones grown in a common garden. Litter from all clones was exposed to each of five environmental conditions: (a) enclosed control in ambient conditions; (b) climate warming of 2.8 °C; (c) increased nitrogen supply; (d) climate warming and increased nitrogen supply; (e) unenclosed control in ambient conditions.

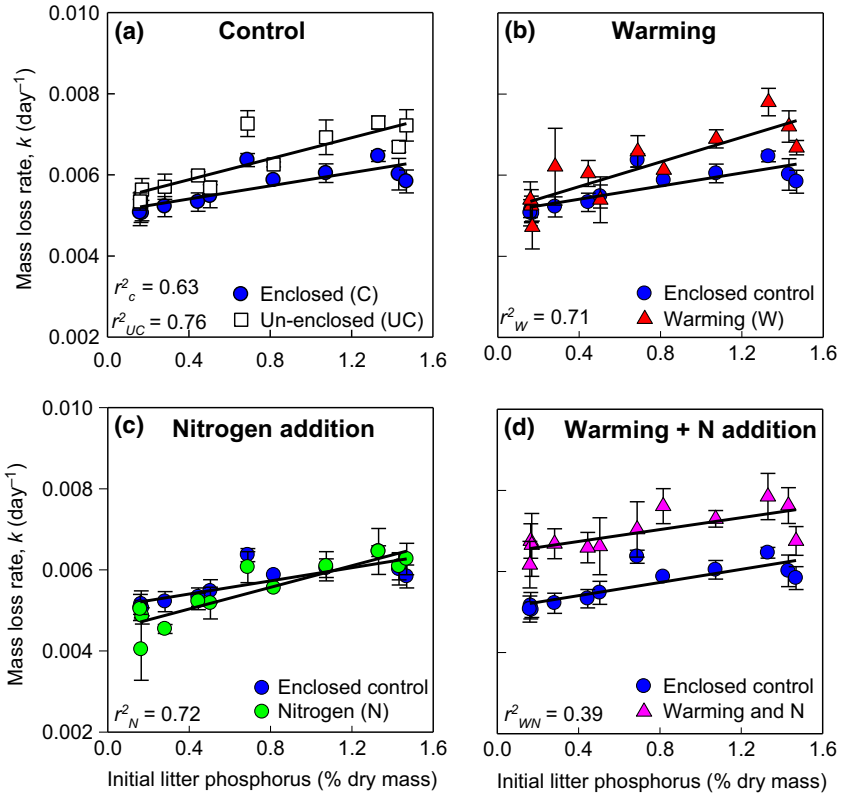

Fig. 3 Consequences of genotypically determined variation in initial phosphorus content of *Phragmites australis* leaf litter on mass loss rate (k) under four global-change scenarios: (a) enclosed (●) and unenclosed (□) controls in ambient conditions; (b) climate warming (▲); (c) increased nitrogen supply (●); and (d) climate warming and increased nitrogen supply (▲). For ease of comparison, the enclosed control is repeated in all panels.

Table 1 Akaike's information criterion (AIC) values for ANCOVA models with different litter-chemistry traits as a covariate. Lowest AIC values indicate best fit of the model to the data

Covariate	Decomposition	Respiration
PCA1	−2382.8	−771.3
Phosphorus	−2421.8	−807.4
Nitrogen	−2400.7	−790.8
Lignin	−2383.5	−805.1
Cellulose	−2336.6	−786.6

explained more than 60% of the variation in ambient environmental conditions: 63% in enclosed, and 76% in unenclosed control plots (Fig. 3a). Furthermore, the close concordance between litter mass loss in enclosed and unenclosed control plots (Enclosure: $F_{1,211} = 0.9$, $P = 0.34$; Table 2) indicates that the enclosures themselves did not modify environmental conditions in ways that notably influenced mass loss rates. The four-fold variation in phosphorus among genotypes corresponds to a 20% increase in litter mass loss in control enclosures, with high-nutrient litter loosing mass faster

Table 2 Results of ANCOVA showing the effects of five treatments (Trt: unenclosed control, enclosed control, warming, excess nitrogen supply, warming and excess nitrogen supply) and genotypically determined variation in litter phosphorus (P) on litter mass loss and microbial respiration. Planned contrasts show direct and interactive effects of climate warming (W), nitrogen supply (N), and the enclosure. Significant P-values ($P < 0.05$) are presented in bold for clarity

		Litter mass loss		Respiration	
Source of variation	df*	F	P	F	P
Treatment (Trt)	4, 211	**13.0**	**<0.0001**	3.0	**0.01**
Genotype phosphorus (P)	1, 211	**106.3**	**<0.0001**	24.4	**<0.0001**
Trt × P	4, 211	1.4	0.23	0.5	0.71
Warming (W)	1, 211	**27.4**	**<0.0001**	0.5	0.45
Nitrogen supply (N)	1, 211	0.3	0.57	1.2	0.27
W × N	1, 211	**18.8**	**<0.0001**	8.2	**0.005**
W × P	1, 211	**5.4**	**0.02**	0.01	0.94
N × P	1, 211	1.7	0.19	0.2	0.65
W × N × P	1, 211	**3.9**	**0.05**	1.4	0.23
Enclosure	1, 211	0.9	0.34	0.6	0.44

*df, degrees of freedom (numerator, denominator).

than low-nutrient litter (P: $F_{1,211} = 106.3$, $P < 0.0001$; Table 2). The close correlation between litter P and N content indicates, however, that litter N content was an almost equally good predictor (Fig. 1; Table 1).

The two global-change factors tested in our experiment (warming and external nitrogen supply) and genotypic variation in litter chemistry produced complex interactive effects on litter mass loss (W × N × P: $F_{1,211} = 3.9$, $P = 0.05$; Fig. 3; Table 2). The strongest effect occurred when both simulated climate warming and nitrogen supply acted in concert (W × N: $F_{1,211} = 18.8$, $P < 0.0001$; Fig. 3d; Table 2). The two global-change factors combined resulted in an acceleration of litter mass loss across all genotypes (Fig. 3d). This synergistic effect of both global-change factors together could not be predicted from the additive effects of nitrogen supply and warming alone (Fig. 3; Table 2). Warming alone accelerated litter mass loss of high- but not low-nutrient genotypes (W × P: $F_{1,211} = 5.4$, $P = 0.02$; Fig 3b; Table 2), whereas external nitrogen supply alone had no direct effect ($F_{1,211} = 0.3$, $P = 0.57$; Table 2; Fig. 2c).

The response pattern of respiration rate to changes in litter chemistry, warming, and nitrogen loading almost exactly mirrored that of litter mass loss (Figs 3 and 4). Respiration rates consistently increased with increasing litter nutrient concentration ($F_{1,211} = 24.4$, $P < 0.0001$ for P; Table 2; Fig. 4), and warming and nitrogen loading in combination produced the same synergistic effect on respiration (W × N: $F_{1,211} = 8.2$, $P = 0.005$; Fig. 4d) as on mass loss (Fig. 3d). As with litter mass loss, external nitrogen supply had no direct effect on respiration rate ($F_{1,211} = 1.2$, $P = 0.27$; Fig. 4c). However, the responses were not completely consistent in that respiration was not significantly stimulated when the warming treatment in the field was applied alone ($F_{1,211} = 0.05$, $P < 0.45$; Fig. 4b).

Discussion

Here we show that genotypically determined variation in litter chemical traits is an important factor determining rates of litter mass loss in current conditions as well as the sensitivity of this ecosystem process to climate warming. By extension, these findings suggest that loss, or gain, of genetic variation associated with expression of key plant traits could buffer or exacerbate the impact of warming on ecosystem processes in the future. A

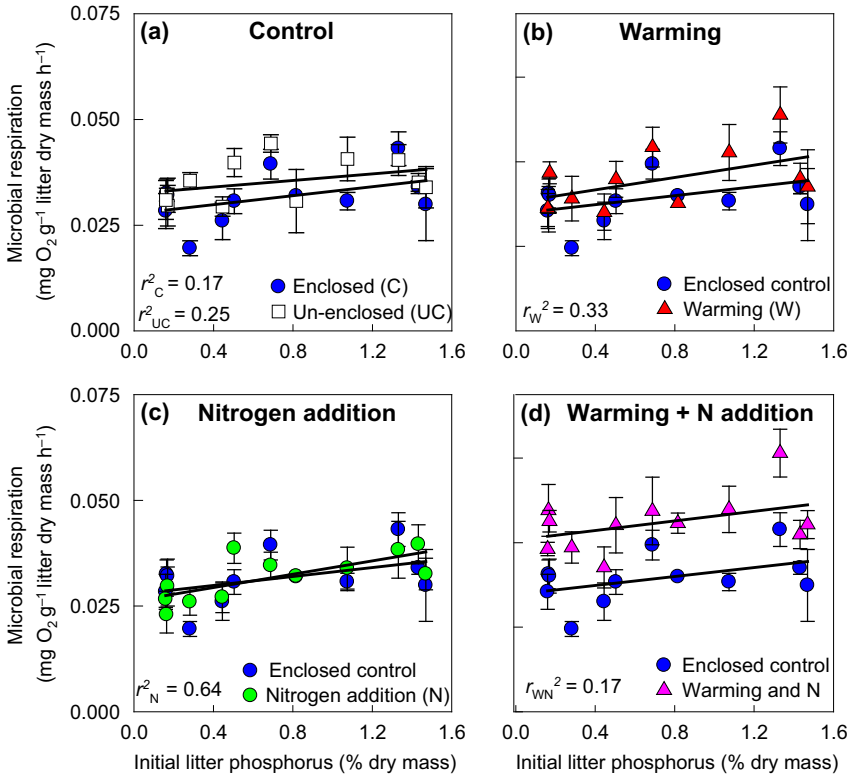

Fig. 4 Consequences of genotypically determined variation in initial phosphorus content of *Phragmites australis* leaf litter on litter-associated microbial respiration under four global-change scenarios: (a) enclosed (●) and unenclosed (□) controls in ambient conditions; (b) climate warming (▲); (c) increased nitrogen supply (●); and (d) climate warming and increased nitrogen supply (▲). For ease of comparison, the enclosed control is repeated in all panels.

second important finding is that multiple factors of global change can have strong synergistic effects. In particular, we found that nitrogen and warming acted in concert to greatly stimulate both litter mass loss and respiration, whereas nitrogen alone had no influence. The close concordance between patterns for mass loss (Fig. 3) and respiration (Fig. 4) indicates that the observed responses to warming and nitrogen deposition are driven by microbial metabolism, and it suggests that the influence of external nitrogen supply on microbial metabolism provides an important context-dependent caveat to hypotheses based on litter chemistry alone.

The observed synergistic changes in litter mass loss and microbial respiration resulting from the two global-change factors in combination could be brought about via several mechanisms. One possibility is that external N supply satisfies increased microbial nitrogen demand when metabolic activity increases at elevated temperatures, but external N supply is not limiting in ambient climatic conditions. An alternative possibility is that increased N supply shifts microbial community structure (Flury & Gessner, 2011) toward species that respond more strongly to warming, as has been observed for communities of fungal litter decomposers in stream microcosms (Dang *et al*., 2009).

Increased external nitrogen supply can both inhibit lignin-degrading enzymes produced by fungi (Carreiro *et al*., 2000) and stimulate microbial metabolism when nitrogen is a limiting element (Bragazza *et al*., 2006; Hyvonen *et al*., 2007). Therefore, the null effect observed here on litter mass loss and respiration during simulated nitrogen loading (Figs 3c and 4c) could have been produced by counteracting mechanisms. Alternatively, it might reflect that phosphorus rather than nitrogen was limiting heterotrophic microbial metabolism, as found elsewhere in wetland and freshwater ecosystems (Sundareshwar *et al*., 2003; Rejmankova & Houdkova, 2006). The strong effect of litter phosphorus content on microbial respiration and litter mass loss (Figs 3 and 4; Table 2) lends some support to the phosphorus limitation hypothesis, although the covariation of litter nitrogen and phosphorus (Fig. 1; Table 1) precludes an unequivocal conclusion about the role of each element.

Notably, our results probably underestimate the potential effects of increased nitrogen supply on decomposition because litter in our experiment was not produced by plants grown under increased nitrogen deposition conditions. Excess nitrogen supply during plant growth can enhance nutrient content of litter when wetland plants assimilate more nutrients than necessary for development (Kröger *et al*., 2007), although plant responses are not always straightforward (Flury & Gessner, 2014). Nonetheless, our clones grown in identical common garden conditions produced a remarkably broad range of litter chemical traits, which allowed for a rigorous test of the consequences of warming and excess nutrient supply on litter mass loss.

The results of our experiment are particularly important for understanding ecosystem responses to global change in ecosystems where genotypes of foundation species show strong intraspecific variation that manifests phenotypically. We found that variation in litter chemistry among genotypes of *P. australis*, a single species grown in a common garden (Fig. 1), can be as great as, or greater than, variation that others have reported among species (Cornwell *et al*., 2008) or in response to environmental variation (Reich & Oleksyn, 2004; Wright *et al*., 2004; Santiago, 2007). This genotypically controlled variation in litter traits proved to have strong effects on litter mass loss and litter-associated microbial respiration in both current and future environmental conditions (Fig. 3). We found that it can also account for inconsistent effects of drivers of global environmental change on ecosystem processes (e.g., no effect vs. positive effects of warming on litter mass loss of plant genotypes that produce low-quality vs. high-quality litter; Fig. 3b; Table 2). Therefore, even when a global-change factor does not influence a given ecosystem process by itself, as was seen with external nitrogen supply in our experiment, it could greatly alter (here accelerate) ecosystem processes if it causes loss of genetic variation associated with key trait values (i.e., low nutrient content; shift to the right in Figs 3c and 4c).

Indeed, the magnitudes of effects on litter mass loss and respiration caused by genotypic variation in our experiment exceeded those produced by either warming or external nitrogen supply alone (genotypic variation vs. treatment in panels B and C: Figs 3 and 4; Table 2); only effects of warming and nitrogen supply in combination surpassed the magnitude of effects that could be produced by loss of genetic variation (genotypic variation vs. treatment in panel D: Figs 3 and 4; Table 2). Thus, if global-change factors accelerate decomposition, as we found for climate warming and excess nitrogen supply together, concomitant loss of genetic variation associated with key traits (e.g., low-nutrient genotypes) could exacerbate the impact of global change on ecosystems (shift to the right and warming + nitrogen effect in Figs 3d and 4d).

Given the importance of intraspecific trait variation of organisms for ecosystem processes (Hooper *et al*., 2012), the conservation of genetic diversity, particularly of foundation species such as *P. australis*, is likely to be a useful strategy to mitigate impacts of global change on ecosystem functioning (Reusch *et al*., 2005). Moreover, informed planting of genotypes with key traits

may reduce or counteract the impact of climate change on restored ecosystems (Kettenring *et al.*, 2014). However, to fully realize the potential of this idea, solid information about the spatial distribution, and potential range shifts of genotypic traits that influence ecosystem processes is needed (Bailey *et al.*, 2013). There is evidence that trait variation can be selected for by both broad-scale abiotic (Reich & Oleksyn, 2004; Wright *et al.*, 2004; Santiago, 2007) and biotic (Pennings & Silliman, 2005; Pennings *et al.*, 2007) environmental gradients, as also found among our *P. australis* clones taken at 12 different locations along a 2100 km latitudinal gradient (Fig. 1). It must be kept in mind, however, that our plant genotypes showed substantial variation also *within* regions (Fig. 1). This observation is consistent with evidence suggesting that the interplay of selective forces determining plant trait values can be complex (Albert *et al.*, 2010). Nonetheless, if genetic divergence among populations influences the phenotypic traits that are key for ecosystems processes, as found here, then examining the spatial distribution of the pertinent genotypic traits in combination with multiple global-change factors may hold the key to understanding variation in ecosystem responses to global environmental change in the future.

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