Global Change Biology

Global Change Biology (2012) **18**, 2617–2625, doi: 10.1111/j.1365-2486.2012.02685.x

The effect of experimental warming and precipitation change on proteolytic enzyme activity: positive feedbacks to nitrogen availability are not universal

EDWARD R. BRZOSTEK*[1], JOHN M. BLAIR†[2], JEFFREY S. DUKES‡, SERITA D. FREY§, SARAH E. HOBBIE¶, JERRY M. MELILLO‖, ROBERT J. MITCHELL**, ELISE PENDALL††, PETER B. REICH‡‡, GAIUS R. SHAVER‖, ARTUR STEFANSKI‡‡, MARK G. TJOELKER§§ and ADRIEN C. FINZI*

*Department of Biology, Boston University, Boston, Massachusetts 02215, USA, †Division of Biology, Kansas State University, Manhattan, Kansas 66502, USA, ‡Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907, USA, §Department of Natural Resources, University of New Hampshire, Durham, New Hampshire 03824, USA, ¶Department of Ecology, Evolution and Behavior, University of Minnesota, St. Paul, Minnesota 55108, USA, ‖Marine Biological Laboratory, The Ecosystems Center, Woods Hole, Massachusetts 02543, USA, **The Joseph W. Jones Ecological Research Center at Ichauway, Newton, Georgia 39870, USA, ††Department of Botany, University of Wyoming, Laramie, Wyoming 82071, USA, ‡‡Department of Forest Resources, University of Minnesota, St. Paul, Minnesota USA, §§Hawkesbury Institute for the Environment, University of Western Sydney, Penrith, NSW 2751, Australia

Abstract

Nitrogen regulates the Earth's climate system by constraining the terrestrial sink for atmospheric CO_2. Proteolytic enzymes are a principal driver of the within-system cycle of soil nitrogen, yet there is little to no understanding of their response to climate change. Here, we use a single methodology to investigate potential proteolytic enzyme activity in soils from 16 global change experiments. We show that regardless of geographical location or experimental manipulation (i.e., temperature, precipitation, or both), all sites plotted along a single line relating the response ratio of potential proteolytic activity to soil moisture deficit, the difference between precipitation and evapotranspiration. In particular, warming and reductions in precipitation stimulated potential proteolytic activity in mesic sites – temperate and boreal forests, arctic tundra – whereas these manipulations suppressed potential activity in dry grasslands. This study provides a foundation for a simple representation of the impacts of climate change on a central component of the nitrogen cycle.

Keywords: global change, organic nitrogen, proteolytic enzymes, soil nitrogen cycle, soil organic-matter decomposition

Received 27 October 2011; revised version received 10 February 2012 and accepted 21 February 2012

Introduction

Proteolytic enzymes depolymerize protein, a large pool of organic nitrogen (N) in soil organic matter (SOM, Schulten & Schnitzer, 1998), into amino acids. The activity of proteolytic enzymes is a principal driver of the within-system cycle of soil N and the amino acids produced by proteolytic enzymes contribute significantly to the N economy of plants and microbes (Schimel & Bennett, 2004; Finzi & Berthrong, 2005; Gallet-Budynek *et al*., 2009; Nasholm *et al*., 2009). Changes in soil temperature and moisture as result of global change have the potential to impact proteolytic enzyme activity by altering the production of enzymes and substrates (Melillo *et al*., 2002; Allison *et al*., 2010a). Given the importance of proteolytic enzymes to N cycling and primary production, it is essential to develop an empirical understanding of their response to warming and changes in precipitation so that this understanding can eventually be incorporated into current ecosystem models.

Most ecosystem models predict that N mineralization and the availability of soil N to support primary production increases with increasing temperature in nearly all biomes (TEM, Raich *et al*., 1991; CLM, Thornton & Rosenbloom, 2005; CENTURY, Parton *et al*., 1993). In these models, the rate of N mineralization is determined by the rate of C mineralization in pools with different turnover times, temperature and moisture sensitivities, and C : N ratios. This linear dependence assumes that the decomposition of N from SOM follows that of C. Recent data from arctic and temperate

[1]Correspondence: Edward R. Brzostek, Department of Biology, Indiana University, 701 Kirkwood Ave, Bloomington, IN 47405, USA, tel. 617 650 7830, fax 812 855 1661,
e-mail: edbrzost@indiana.edu

[2](Correction added after online publication 09/04/2012: The authors' names and affiliations were added accordingly.)

forest biomes show that the activity of N-degrading enzymes in the soil are less responsive to temperature than those that degrade soil C (Wallenstein *et al*., 2009; Brzostek & Finzi, 2011), suggesting that current generation of models may overestimate the availability of N to support primary production. In defense of models, however, there is limited data to parameterize these models, and the empirical basis for enzymatic responses to climate change is only now emerging (e.g., Schimel & Weintraub, 2003; Allison *et al*., 2010b; Davidson *et al*., 2012; Wang *et al*., 2012).

Results from short-term laboratory experiments suggest that among-biome differences in climate will significantly impact the response of proteolytic enzyme activity to rising temperature and changes in precipitation. In the short term, increases in temperature and soil moisture have been shown to enhance the activity of enzymes by increasing the collision frequency and solubility of enzymes and substrates (e.g., Wallenstein *et al*., 2009; Allison *et al*., 2010a), with the largest temperature effects observed in soils collected during the winter and early spring when they are cold (e.g., Fenner *et al*., 2005; Wallenstein *et al*., 2009; Brzostek & Finzi, 2012). Further, low soil moisture has been shown to limit the positive response of enzyme activity to increasing temperatures (Zak *et al*., 1999; Davidson *et al*., 2012). This evidence suggests that warming will have the greatest impact on proteolytic enzyme activity in cold, mesic biomes, whereas changes in soil moisture will control responses in warmer, xeric biomes.

Over longer time scales, the response of proteolytic enzyme activity to changes in soil temperature and moisture may differ substantially from laboratory manipulations where enzyme pool size and substrate availability are held constant. Initial increases and subsequent declines in SOM decomposition in response to warming (Melillo *et al*., 2002; Bradford *et al*., 2008) suggest that low substrate availability or reduced enzyme production can lead to declines in enzyme activity with increasing temperature (Allison *et al*., 2010b). Thus, even if proteolytic enzymes were highly sensitive to short-term changes in temperature and moisture, low levels of enzyme and substrate could limit enzymatic responses to global change in many ecosystems.

The aggregate effects of warming and precipitation change on microbial processes are often linked through their impact on the water balance of ecosystems (Knapp *et al*., 2002, 2008; Austin *et al*., 2004; Arnone *et al*., 2008). Climate change is therefore likely to result in a continuum of enzymatic responses across biomes; though to date there have been no systematic evaluations of such changes in the N cycle. In this study, we use soils from 16 existing global change experiments to understand the long-term effect of climate change on the potential activity of proteolytic enzymes (Table 1). We tested two hypotheses: (1) warming increases potential proteolysis in mesic sites and decreases potential activity in xeric sites and (2) precipitation manipulations that exacerbate large, water balance deficits decrease potential proteolysis, while those that cause reductions in water balance deficits or surpluses increase potential activity. The experiments are located in several North American biomes, ranging from dry grasslands to temperate and boreal forest to arctic tundra. We evaluated changes in potential proteolysis among biomes using a single methodology to measure changes in potential proteolysis in response to experimental manipulations of temperature and/or precipitation.

Materials and methods

Site description and soil collection

We collected soils from 16 global change experiments from 10 different sites across the United States (Table 1). At each site, experiments manipulated soil temperature, precipitation input, or a combination of both factors. The sites were located across a broad latitudinal gradient from arctic tundra (LAT: 68.6N) to lower mid-latitude grasslands and savannah (LAT: 30.6N). Soils were sampled from replicate control and treatment plots between July and September of 2009, near the peak of the growing season at each site. The exception was the N × Warming (HFN) experiment at the Harvard Forest, MA, where soils were sampled in mid-October 2009. There were more warming studies than precipitation-change studies in the data set, allowing for meaningful horizon-specific analyses of enzymatic response to warming. Only mineral-soil samples were available from experiments manipulating precipitation.

Soils were sampled following established protocols at each site. Organic horizons when present and the top 15 cm of mineral soil were collected and analyzed separately. Immediately after coring the soils were shipped or transported on ice to Boston University for analysis. Upon arrival, each soil sample was sieved, roots and rocks removed, and a 30 g subsample frozen at −80 °C. Given the large number of soil samples (>800 cores), it was necessary to store samples at −80 °C prior to the analysis of proteolytic activity. It was not logistically possible to analyze the samples at the same rate as they were received from the different sites.

Potential proteolytic enzyme activity

We assayed potential proteolytic enzyme activity following a method modified from Watanabe & Hayano (1995) and Lipson *et al*. (1999). To compare rates of proteolysis among experiments, we warmed the soils for 4 h to a common laboratory temperature of 23 °C. Initial and incubated subsamples of soil (2–3 g) received 10 ml of a 0.5 mM sodium acetate buffer (pH 5.0) with a small volume of toluene (400 μl) added to inhibit microbial uptake. After these reagent additions, the initial

Table 1 Site locations, global change manipulations, and climate

Site	Abbrev.	Location	Biome	Manipulations	LAT	LONG	MAT (°C)	MAP (mm)
Toolik-Moist Acidic Tundra	T-MAT	Toolik Lake, AK	Tundra	Warming	68.6	−149.6	−8.6	328
Toolik Nonacidic Tundra	T-NAT	Toolik Lake, AK	Tundra	Warming	68.6	−149.6	−8.6	328
Toolik-Shrub	T-SHB	Toolik Lake, AK	Tundra	Warming	68.6	−149.6	−8.6	328
Toolik-Wet Sedge Grass	T-WSG	Toolik Lake, AK	Tundra	Warming	68.6	−149.6	−8.6	328
Boreal Forest Warming at a Ecotone in Danger	B4W-1	Cloquet, MN	Boreal Forest	Warming	46.7	−92.5	4.6	807
B4WarmED	B4W-2	Ely, MN	Boreal Forest	Warming	47.9	−91.8	1.4	739
Harvard Forest-Prospect Hill	HF1	Petersham, MA	Temperate Forest	Warming	42.5	−72.2	7.8	1172
Harvard Forest-Barre Woods	HF2	Petersham, MA	Temperate Forest	Warming	42.5	−72.2	7.8	1172
Harvard Forest-N × Warming	HFN	Petersham, MA	Temperate Forest	Warming	42.5	−72.2	7.8	1172
Boston Area Climate Experiment	BACE	Waltham, MA	Temperate Old Field	Warming, ↑↓ Precipitation	42.4	−71.2	10.9	1104
Long Leaf Pine Irrigation	LLP	Newton, GA	Coastal Pine Forest	↑ Precipitation	31.3	−84.4	19.1	1356
Biodiversity, CO_2, and Nitrogen	BioCON	Cedar Creek, MN	Grassland	↓ Precipitation	45.4	−93.3	6.27	796
Prairie Heating and CO_2 Experiment	PHACE	High Plains, WY	Prairie	Warming, ↑ Precipitation	41.1	−104.8	7.2	384
Rainfall Manipulation Plot Study	RAMPS	Konza Prairie, KS	Prairie	Warming, precipitation timing	39.1	−96.6	12.7	872
Konza Irrigation Transect	KIT	Konza Prairie, KS	Prairie	↑ Precipitation	39.1	−96.6	12.7	872
Warming and Rainfall Manipulation Experiment	WARM	College Station, TX	Oak-savannah	Warming, precipitation timing	30.6	−96.4	20.3	981

LAT, latitude; LONG, longitude; MAT, mean annual temperature; MAP, mean annual precipitation.

samples were treated with 3 ml of a trichloroacetic acid solution to halt the activity of proteolytic enzymes. The remaining subsamples were incubated for 4 h and then treated with the same trichloroacetic acid solution (Finzi & Berthrong, 2005; Rothstein, 2009). The concentration of amino acids in the initial and incubated samples was quantified using the *o*-phthaldialdehyde and *β*-mercaptoethanol method (Jones *et al*., 2002). Concentrations of amino acid N were determined by comparing the fluorescence of the samples relative to a standard curve composed of glycine. Potential activity was calculated as the difference between amino acid concentrations in the incubated and initial samples (Brzostek & Finzi, 2011). We acknowledge that by assaying proteolytic enzyme activity at common temperature, moisture, and pH conditions, we did not specifically measure *in situ* rates of activity. In particular, the *in situ* activity in soils from very cold and very dry sites is likely much lower than the activity reported here. By holding these other factors constant, however, this assay measures a potential enzyme activity where only enzyme pool size and the availability of protein substrates varies between treatments.

Statistical analysis

We used meta-analysis to investigate the response of proteolytic enzymes to manipulations of soil temperature and precipitation inputs across the different experiments. Meta-analysis provides a quantitative statistical approach for synthesizing the results of multiple independent experiments (e.g., Rustad *et al*., 2001; Knorr *et al*., 2005; Treseder, 2008). We used meta-analysis instead of multiple independent ANOVA analyses for each experiment because this approach would preclude a quantitative, cross-site estimate of the magnitude of the response of proteolytic enzymes to temperature and precipitation change (Rosenberg *et al*., 2000). Potential proteolytic activity in the organic horizon was analyzed separately from that in the mineral-soil horizon, because these horizons

Table 2 Ambient and experimentally altered growing-season soil moisture deficits

	Growing-season P-PET (mm)				
Site	Ambient	Warming	↑ Precipitation	↓ Precipitation	Warming × ↓ Precipitation
Toolik Lake (4 exp.)	−144.8	−163.6			
B4W-1	−24.1	−65.0			
B4W-2	10.4	−25.7			
Harvard Forest (3 exp.)	−43.8	−135.9			
BACE	−147.1	−188.9	−24.5	−269.6	
LLP	−82.8		47.8		
BioCON	−41.2			−141.2	
PHACE	−182.4	−213.0	−102.4		
KIT	−135.2		164.8		
RaMPS	−135.2	−199.9		−135.2	−199.9
WaRM	−348.5	−383.2		−433.2	−552.9

P-PET, precipitation (mm) minus potential evapotranspiration (mm).

are functionally distinct and proteolytic activities differ by an order of magnitude or more (e.g., Rothstein, 2009; Brzostek & Finzi, 2011; Reiskind *et al.*, 2011).

For each site, we performed a weighted meta-analysis that factors in the sample size and variability in the responses for each experimental observation (META-WIN Version 2.1; Rosenberg *et al.*, 2000). For each study, we calculated the effect size of a given treatment by calculating the natural log of the response ratio [i.e., ln(RR)], defined here as the mean of the potential proteolytic rate in the treatment divided by the mean rate in the control plot. Values of ln(RR) > 0 indicate stimulatory effects, and values <0, inhibitory effects. For each ln(RR), we used bootstrapping, a nonparametric approach, to calculate 95% confidence intervals (CI). CIs that did not overlap zero indicate a significant treatment response.

One assumption of meta-analysis is that all observations are independent (Rosenberg *et al.*, 2000). Recently, Hungate *et al.* (2009) highlighted how different definitions of what constitutes an independent observation can influence the results of meta-analysis. We adopted their recommended and conservative suggestion for independent observations by calculating a single ln(RR) for experiments that had multiple treatment levels but only a single set of control plots (e.g., BACE, B4W 1 & 2, PHACE).

In addition to experiment-level responses, we used meta-analyses to examine responses across experiments. We calculated the grand mean and the bias corrected bootstrapped CIs for the ln(RR) for the response of potential proteolytic activity to the warming and precipitation manipulations (Adams *et al.*, 1997). We used a categorical random effects model to test for differences in potential proteolytic activity among biomes and warming methods (Rustad *et al.*, 2001).

Finally, weighted regression analysis was used to investigate the relationship between the response of potential proteolytic activity with the duration and magnitude of the experimental treatments and the climate at each site. Weighted regression analyses were performed in META-WIN 2.1

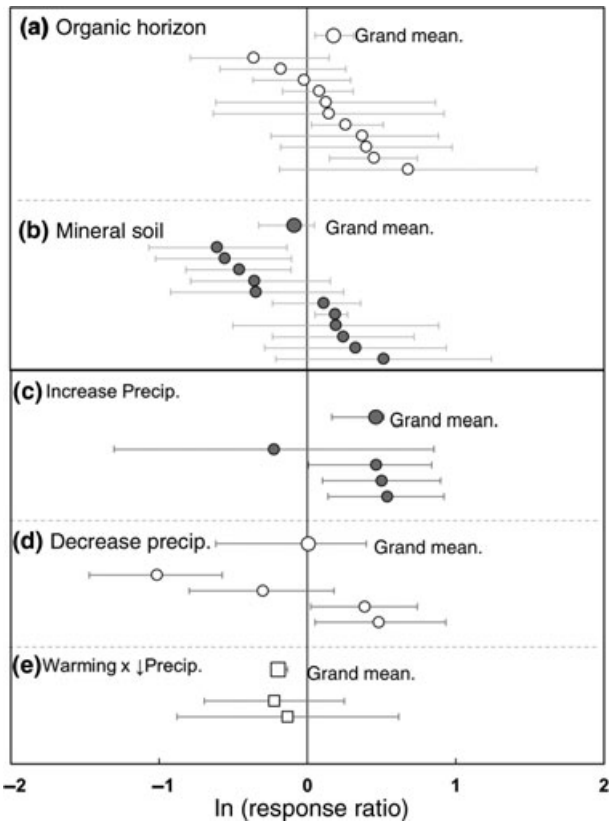

Fig. 1 Response of potential proteolytic enzyme activity to experimental warming in (a) organic horizons (N = 11) and (b) mineral soils (N = 11) and to (c–e) precipitation manipulations (N = 4, ↑&↓ Precip.; n = 2, Warming by ↓ Precip.). For each response, the effect sizes, ln(RR)s, and corresponding 95% CIs are plotted for each independent experimental observation and also for the grand mean of the response of each treatment.

Table 3 Mean ± 1 SEM of potential proteolytic enzyme activity in the control and treatment plots and the effect sizes, ln(RR), with variance (var.) for each experiment

			Mean potential proteolytic activity (μg AA-N g soil^{-1} 4 h^{-1})		Effect size	
Site	Horizon	Manipulations	Control	Treatment	ln(RR)	Var.
T-MAT	OH	Warming	22.83 (1.72)	24.74 (3.61)	0.080	0.027
T-NAT	OH	Warming	28.82 (7.40)	24.13 (3.87)	−0.178	0.092
T-SHB	OH	Warming	19.83 (2.02)	31.09 (5.94)	0.450	0.047
T-WSG	OH	Warming	8.60 (3.38)	6.00 (0.31)	−0.359	0.157
B4W-1	OH	Warming-Closed	38.72 (4.33)	43.91 (10.73)	0.126	0.142
B4W-1	OH	Warming-Open	10.29 (1.93)	11.89 (2.05)	0.145	0.157
B4W-2	OH	Warming-Closed	2.23 (0.52)	3.54 (0.79)	0.399	0.087
B4W-2	OH	Warming-Open	2.49 (0.61)	4.93 (1.81)	0.680	0.195
HF1	OH	Warming	15.66 (2.31)	20.17 (1.16)	0.260	0.025
HF2	OH	Warming	4.29 (0.63)	4.19 (0.67)	−0.022	0.047
HFN	OH	Warming	5.95 (0.77)	8.61 (2.81)	0.370	0.123
B4W-1	MS	Warming-Closed	1.42 (0.42)	1.72 (0.34)	0.194	0.125
B4W-1	MS	Warming-Open	0.81 (0.17)	1.12 (0.22)	0.326	0.097
B4W-2	MS	Warming-Closed	0.34 (0.08)	0.57 (0.20)	0.515	0.137
B4W-2	MS	Warming-Open	0.29 (0.10)	0.37 (0.04)	0.244	0.059
BACE	MS	Warming	1.36 (0.29)	1.52 (0.15)	0.187	0.055
HF1	MS	Warming	3.36 (0.56)	2.36 (0.59)	−0.354	0.089
HF2	MS	Warming	1.88 (0.30)	1.19 (0.19)	−0.458	0.052
HFN	MS	Warming	2.10 (0.52)	1.49 (0.53)	−0.345	0.189
PHACE	MS	Warming	2.75 (0.74)	3.19 (0.93)	0.111	0.325
RaMPS	MS	Warming	3.43 (0.66)	1.87 (0.41)	−0.606	0.084
WaRM	MS	Warming	0.57 (0.19)	0.33 (0.08)	−0.554	0.080
BioCON	MS	↓ Precipitation	1.01 (0.16)	1.63 (0.35)	0.482	0.073
BACE	MS	↓ Precipitation	1.08 (0.16)	1.50 (0.21)	0.387	0.093
RaMPS	MS	↓ Precipitation	3.43 (0.66)	2.55 (0.65)	−0.300	0.103
WaRM	MS	↓ Precipitation	0.57 (0.13)	0.21 (0.03)	−1.016	0.076
PHACE	MS	↑ Precipitation	2.13 (0.98)	1.70 (0.51)	−0.226	0.303
BACE	MS	↑ Precipitation	1.08 (0.16)	1.86 (0.27)	0.501	0.041
KIT	MS	↑ Precipitation	1.31 (0.22)	2.46 (0.38)	0.538	0.059
LLP	MS	↑ Precipitation	0.31 (0.08)	0.50 (0.05)	0.463	0.077
RaMPS	MS	Warming + ↓ Precip.	3.43 (0.66)	3.01 (0.99)	−0.133	0.145
WaRM	MS	Warming + ↓ Precip.	0.57 (0.19)	0.40 (0.04)	−0.224	0.058

using a continuous random effects model. Mean (1971–2000) growing season and annual climate information (MAT, MAP) for each site was obtained from the National Climate Data Center (Table 1 and Supporting Information Table S1). Soil moisture deficit (or surplus) was calculated as the difference between precipitation (P) and potential evapotranspiration (PET) (i.e., P-PET; Liski *et al*., 2003; McCarthy *et al*., 2010). Monthly mean temperatures and latitude were used to calculate PET during the growing season using the Thornthwaite method (Thornthwaite, 1948). We used data from the precipitation and warming manipulations to calculate treatment specific P-PET (Table 2). The details of the experimental treatments for each site are listed in Table S2. We acknowledge that differences between sites in the method of warming may have a modest impact on the accuracy of our P-PET estimate (Aronson & McNulty, 2009). The error in our PET estimates is likely lowest in those sites that use IR heaters, which accurately simulate energy balance changes and highest in those sites that use cables or chambers, which have been shown to decrease soil moisture (Kennedy, 1995; Shaver *et al*., 2000).

Results

Potential proteolytic enzyme activity

The response of potential proteolytic enzyme activity to warming in the organic and mineral-soil horizons was highly variable (Fig. 1, Table 3). Overall, experimental warming led to a significant 18% increase in potential proteolytic activity in organic horizons (Fig. 1a). Warming did not have a significant overall effect on potential proteolytic activity in the mineral soil (Fig. 1b). There were no significant differences in potential proteolytic activity between horizons, among biomes or in response to different methods of warming.

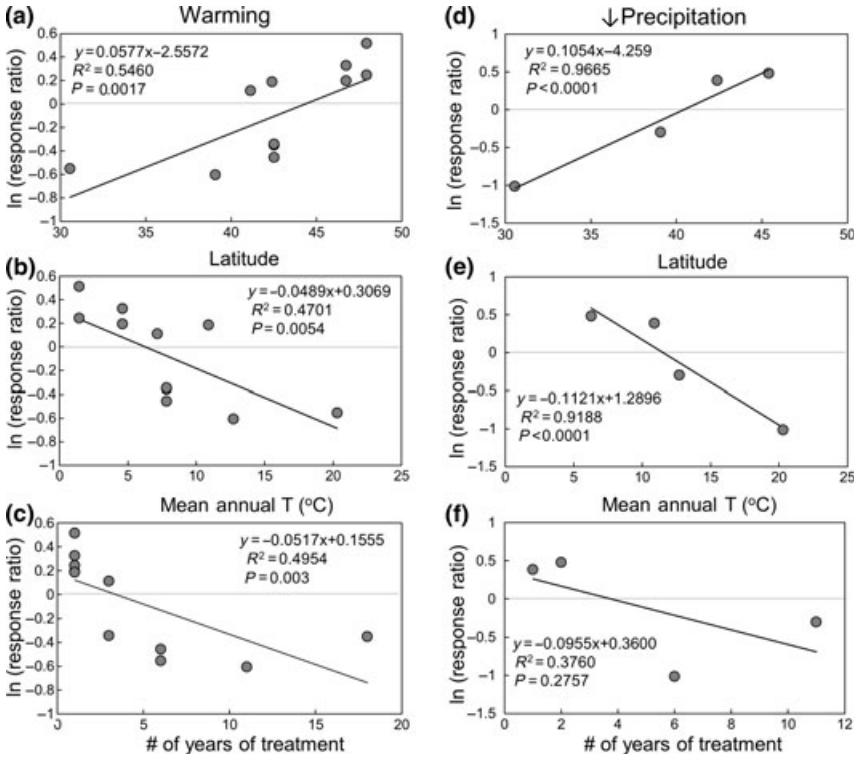

Fig. 2 Left panels show the relationship between the response of potential proteolytic enzyme activity to warming in the mineral soil with (a) latitude, (b) MAT, and (c) # of years of experimental treatment (N = 11). Right panel shows the relationship between the response of proteolytic enzyme activity to decreased soil moisture treatments in the mineral soil with (d) latitude, (e) MAT, and (f) # of years of experimental treatment (N = 4). Lines are the weighted regression through all points.

In the mineral-soil horizon, among-study variations in the response ratio of potential proteolytic activity was positively correlated with latitude (R^2 = 0.55, P < 0.002, Fig. 2a) and negatively correlated with mean annual temperature (R^2 = 0.47, P < 0.01, Fig. 2b). The response to warming declined significantly with the number of years of experimental treatment (R^2 = 0.50, P < 0.005, Fig. 2c). There were no significant relationships between these variables and potential proteolytic activity in response to warming in the organic horizon.

Experimental increases in precipitation significantly increased potential proteolytic activity (Fig. 1c), whereas decreases in precipitation had both positive and negative effects on potential enzyme activity (Fig. 1d). Variation in the response ratio of potential proteolytic activity among studies was negatively correlated with temperature and PET, but not precipitation (Table S3). As with warming, the response ratio of potential proteolytic activity was positively correlated with latitude (R^2 = 0.97, P < 0.0001, Fig. 2d), negatively correlated with mean growing-season temperature (R^2 = 0.92, P < 0.0001, Fig. 2e), and tended to decline with length of treatment (R^2 = 0.38, P = 0.28, Fig. 2f).

There were only two experiments that both warmed and manipulated precipitation (RAMPS and WARM; Table 1). In these studies, warming and changes in the timing of precipitation decreased potential proteolytic activity (Fig. 1e). Across all manipulations, however, treatment induced changes in the magnitude of the soil moisture deficit (i.e., growing-season P-PET; Table 2) best predicted the variability in enzymatic response (Fig. 3a). Potential proteolytic activity in the mineral soil decreased linearly with increasing soil moisture deficit (R^2 = 0.47, P < 0.0001; Fig. 3a). There was no such relationship in the organic horizon (Fig. 3b). Given this strong relationship between P-PET and the response of proteolytic enzymes in the mineral soil, we used a weighted multiple regression to further examine this relationship. Ambient P-PET conditions combined with the magnitude and direction of the change in P-PET provided a good prediction of the response of potential proteolytic activity to global change (R^2 = 0.43, P < 0.003, Table S3).

Discussion

Nitrogen limitation of primary production is widespread in the biosphere and N, in part, regulates the Earth's climate system by constraining terrestrial uptake of atmospheric CO_2 (Vitousek & Howarth, 1991;

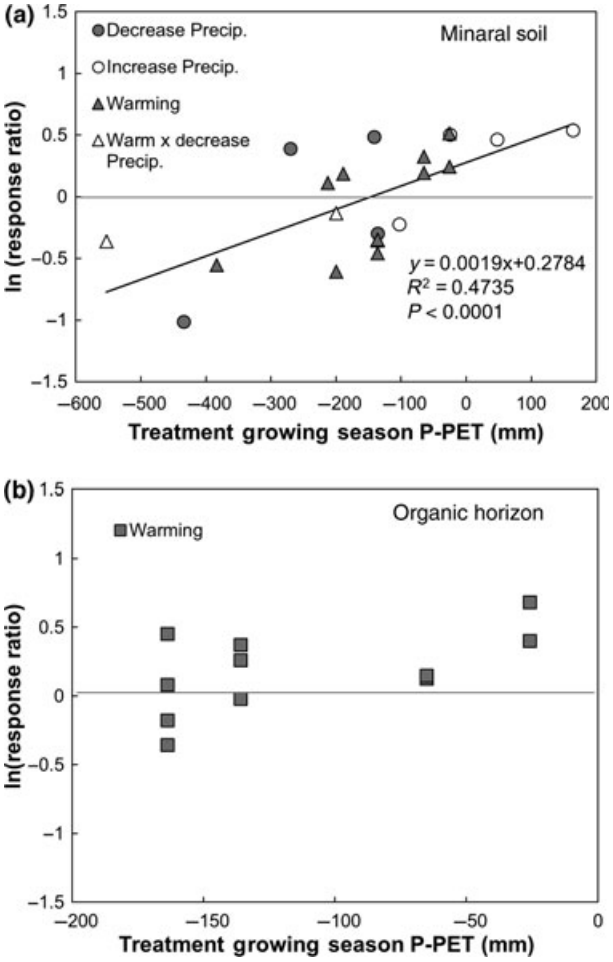

Fig. 3 Relationships between the responses of potential proteolytic enzyme activity to warming and precipitation manipulations in the (a) mineral soil (N = 21) and to warming in the (b) organic horizon (N = 11) with the treatment induced growing-season P-PET. Line in (a) is the weighted regression through all points.

Melillo *et al*., 1993; Hungate *et al*., 2003). Here, we show that the depolymerization of organic N, primarily in the form of protein, is highly sensitive to climate change. The experimental treatments altered the water balance of each ecosystem. The rate of potential proteolysis in the treatment relative to control plots declined linearly as the magnitude of the growing-season soil moisture deficit increased (i.e., P-PET became more negative; Fig. 3a), and all biomes plotted along the same line. This suggests the existence of a single relationship between climate and proteolytic enzymes and provides a foundation for a simple representation of climate change impacts on a key component of the terrestrial N cycle.

There was a relatively narrow range of soil moisture deficit (-200 < P-PET < -100) above which potential proteolytic rates tended to increase and below which they tended to decline (Fig. 3a). In the experimental treatments that reduced soil moisture below this threshold, it is likely that the diffusion of substrates and enzymes decreased, leading to a decline in the enzymatic return of N that limited proteolytic enzyme production and potential activity. (Zak *et al*., 1999; Allison *et al*., 2010a; Davidson *et al*., 2012). Above this threshold, warming and adequate soil moisture stimulated potential proteolytic activity, which in this case is likely to reflect some combination of an increase in substrate supply, and an increase in enzyme production by a larger or more active microbial biomass (Fig. 3a). Given that we did not measure extractable protein concentrations or microbial biomass, we cannot definitively argue which of these processes, or others, explain the observed response. Regardless, this analysis indicates that relatively subtle variations in climate have the potential to substantially alter the activity of proteolytic enzymes in mid- and high-latitude ecosystems.

In the mineral-soil horizon, the increase in potential proteolytic activity in response to warming was negatively correlated with MAT and positively correlated with latitude (Fig. 2a, b). The correlation with latitude was, in part, a reflection of the distribution of the global change experiments. The lower latitude sites tended to be in grasslands where warming enhanced the soil moisture deficit below the threshold of -200 mm. The higher latitude sites tended to be in cold, mesic forests where warming did not move the soil moisture deficit below this threshold. However, given the low ambient temperatures at these sites, warming had a proportionately larger effect on temperature that appeared to stimulate potential proteolytic activity (Table 1, Table S3). These considerations do not, however, compromise the major conclusion reported here. The two largest stimulations in potential proteolytic activity were measured in response to irrigation at the Konza prairie site (KIT: treatment P-PET = 165 mm) and in the lower latitude long leaf pine study (LLP: treatment P-PET = 48 mm), indicating that mid-latitude grasslands and warm-temperate forests respond to soil moisture deficit in a manner that is quantitatively similar to higher latitude forests.

The response of mineral-soil potential proteolytic activity to warming declined with the duration of experimental treatment (Fig. 2c). One interpretation of this relationship is that there is an initial increase in proteolytic activity followed by a decline in activity as readily available substrates are depleted (Melillo *et al*., 2002). However, three of the five experiments that anchor this relationship were from warming experiments of variable duration at the Harvard Forest.

Whether collected following three (HFN), six (HF2), or 18 years (HF1) of experimental treatment, potential proteolytic activity declined to a similar degree with warming (Table 3). The similarity in response regardless of experimental duration and their plotting on the cross-biome line (Fig. 3a) suggest that the negative correlation between potential proteolytic activity and years of treatment is less important than spatial or experimental variations in climate.

The sites with organic horizons in this study were mid- and high-latitude forests and tundra (Table 1). In only 3 of the 11 studies did potential proteolytic activity in the organic horizon decline in response to warming (Table 3); overall, warming significantly increased potential proteolytic activity in this horizon (Fig. 1a). Experimental warming of the organic horizon did not result in P-PET values below the threshold seasonal soil moisture deficit described above (Table 2). Rather, it appears that any potential, negative effect of warming on soil moisture deficit was offset by the positive effect of warming on potential proteolytic activity. The consistent positive response to warming is likely driven by high protein substrate concentrations that are characteristic of organic horizons (Berthrong & Finzi, 2006; Reiskind *et al.*, 2011).

The results of this study are consistent with an earlier meta-analysis of N mineralization responses to warming in boreal and arctic ecosystems (Rustad *et al.*, 2001); both suggest that warming will increase the rate of N cycling in high-latitude soils. In contrast to Rustad *et al.* (2001), who found little relationship between potential N mineralization, MAT, or MAP, we found that variation in potential proteolytic activity was highly correlated with among-site variations in climate and the impact of climate manipulations on seasonal soil moisture deficit. Moreover, other studies have shown a soil moisture sensitivity of net N mineralization (Cassman & Munns, 1980; Emmett *et al.*, 2004). Analysis of soil moisture deficit for the sites in Rustad *et al.* (2001) suggests an explanation for the apparent discrepancy between studies. The seasonal soil moisture deficit with warming in the organic-matter rich soils studied by Rustad *et al.* (2001) ranged from −100 to −230 mm, well within the range of positive responses observed in the organic horizons in this study (Fig. 3b).

Amino acids released from protein substrate by proteolytic enzymes contribute to terrestrial productivity and serve as substrates for mineralization and nitrification (Chapin *et al.*, 1993; Nasholm *et al.*, 1998; Jones & Kielland, 2002; Gallet-Budynek *et al.*, 2009). As such, amino acid cycling is a key component of the terrestrial N cycle. The results of this study suggest that regional-scale changes in temperature and precipitation can control the magnitude and direction of the N-cycle responses to climate change. Understanding regional climate change is therefore essential to understanding the global-scale consequences of rising concentrations of radiatively active trace gasses on terrestrial productivity.

Acknowledgements

We would like to thank Colin Averill, Joy Cookingham, Verity Salmon, Poliana Lemos, Marc-Andre Giasson, Alison Greco and Winston MacDonald for laboratory assistance and Janet Chen for field assistance (PHACE). Funding for the work presented in this paper was provided by grants from the National Science Foundation (NSF, DEB-0743564, DEB-1011479) and a Northern Forest Scholar fellowship from the Northeastern States Research Cooperative, a joint program of the University of Vermont, the University of Maine and the Northern Research Station, USDA Forest Service to E.R.B. The following sources supported core operation for the global change experiments: (1) WaRM: Department of Energy's (DOE) Office of Science (BER) through the Southeastern Regional Center of the National Institute for Climatic Change Research, DOE-BER National Institute of Global Environmental Change and Texas AgriLife Research; (2) BioCON & B4Warmed: DOE-BER National Institute for Climate Change Research (2 awards), and NSF programs in Biocomplexity, Long-term Ecological Research (LTER, 2 awards), Division of Environmental Biology (DEB) and Long-term Research in Environmental Biology (LTREB); (3) PHACE: US Department of Agriculture (USDA)-Agricultural Research Service Climate Change, Soils & Emissions Program and Extension Service Soil Processes Program; (4) Arctic Sites T-MAT, -NAT, -SHB, -WSG: NSF programs in DEB, Office of Polar Programs and the Arctic LTER; (5) HFN: NSF Faculty Early Career Development Award to Serita Frey and Harvard Forest LTER; (6) HF-1, HF-2: NSF Harvard Forest LTER and DOE-BER Northeastern Regional Center of the National Institute for Climatic Change Research; (7) RaMPs: USDA, DOE-BER Northeastern Regional Center of the National Institute for Climatic Change Research and NSF LTREB; (8) KIT: NSF Konza Prarie LTER to Kansas State University; (9) BACE: NSF, Division of Environmental Biology and DOE-BER Northeastern Regional Center of the National Institute for Climatic Change Research; (10) DOE-BER National Institute for Climate Change Research. (Correction added after online publication 09/04/2012: Acknowledgements section has been updated by the authors.)

References

Adams DC, Gurevitch J, Rosenberg MS (1997) Resampling tests for meta-analysis of ecological data. *Ecology*, **78**, 1277–1283.

Allison SD, Weintraub MN, Gartner TB, Waldrop MP (2010a) Evolutionary-economic principles as regulators of soil enzyme production and ecosystem function. In: *Soil Enzymology* (eds Shukla GC, Varma A), pp. 229–243. Springer-Verlag, New York.

Allison SD, Wallenstein MD, Bradford MA (2010b) Soil-carbon response to warming dependent on microbial physiology. *Nature Geoscience*, **3**, 336–340.

Arnone JA, Verburg PSJ, Johnson DW *et al.* (2008) Prolonged suppression of ecosystem carbon dioxide uptake after an anomalously warm year. *Nature*, **455**, 383–386.

Aronson EL, McNulty SG (2009) Appropriate experimental ecosystem warming methods by ecosystem, objective, and practicality. *Agricultural and Forest Meteorology*, **149**, 1791–1799.

Austin AT, Yahdjian L, Stark JM *et al.* (2004) Water pulses and biogeochemical cycles in arid and semiarid ecosystems. *Oecologia*, **141**, 221–235.

Berthrong ST, Finzi AC (2006) Amino acid cycling in three cold-temperate forests of the northeastern USA. *Soil Biology & Biochemistry*, **38**, 861–869.

Bradford MA, Davies CA, Frey SD *et al.* (2008) Thermal adaptation of soil microbial respiration to elevated temperature. *Ecology Letters*, **11**, 1316–1327.

Brzostek ER, Finzi AC (2011) Substrate supply, fine roots and temperature control proteolytic enzyme activity in temperate forest soils. *Ecology*, **92**, 892–902.

Brzostek ER, Finzi AC (2012) Seasonal variation in the temperature sensitivity of proteolytic enzyme activity in temperate forest soils. *Journal of Geophysical Research, Biogeosciences*, **117**, G01018.

Cassman KG, Munns DN (1980) Nitrogen mineralization as affected by soil-moisture, temperature, and depth. *Soil Science Society of America Journal*, **44**, 1233–1237.

Chapin FS, Moilanen L, Kielland K (1993) Preferential use of organic nitrogen by a nonmycorrhizal arctic sedge. *Nature*, **361**, 150–153.

Davidson EA, Samanta S, Caramori SS, Savage K (2012) The Dual Arrhenius and Michaelis-Menten (DAMM) kinetics model for decomposition of soil organic matter at hourly to seasonal time scales. *Global Change Biology*, **18**, 371–384.

Emmett BA, Beier C, Estiarte M *et al.* (2004) The response of soil processes to climate change: results from manipulation studies of shrublands across an environmental gradient. *Ecosystems*, **7**, 625–637.

Fenner N, Freeman C, Reynolds B (2005) Observations of a seasonally shifting thermal optimum in peatland carbon-cycling processes; implications for the global carbon cycle and soil enzyme methodologies. *Soil Biology & Biochemistry*, **37**, 1814–1821.

Finzi AC, Berthrong ST (2005) The uptake of amino acids by microbes and trees in three cold-temperate forests. *Ecology*, **86**, 3345–3353.

Gallet-Budynek A, Brzostek E, Rodgers VL, Talbot JM, Hyzy S, Finzi AC (2009) Intact amino acid uptake by northern hardwood and conifer trees. *Oecologia*, **160**, 129–138.

Hungate BA, Dukes JS, Shaw MR, Luo YQ, Field CB (2003) Nitrogen and climate change. *Science*, **302**, 1512–1513.

Hungate BA, van Groenigen KJ, Six J *et al.* (2009) Assessing the effect of elevated carbon dioxide on soil carbon: a comparison of four meta-analyses. *Global Change Biology*, **15**, 2020–2034.

Jones DL, Kielland K (2002) Soil amino acid turnover dominates the nitrogen flux in permafrost-dominated taiga forest soils. *Soil Biology & Biochemistry*, **34**, 209–219.

Jones DL, Owen AG, Farrar JF (2002) Simple method to enable the high resolution determination of total free amino acids in soil solutions and soil extracts. *Soil Biology & Biochemistry*, **34**, 1893–1902.

Kennedy AD (1995) Simulated climate-change- Are passive greenhouses a valid microcosm for testing the biological effects of environmental perturbations. *Global Change Biology*, **1**, 29–42.

Knapp AK, Fay PA, Blair JM *et al.* (2002) Rainfall variability, carbon cycling, and plant species diversity in a mesic grassland. *Science*, **298**, 2202–2205.

Knapp AK, Beier C, Briske DD *et al.* (2008) Consequences of more extreme precipitation regimes for terrestrial ecosystems. *BioScience*, **58**, 811–821.

Knorr M, Frey SD, Curtis PS (2005) Nitrogen additions and litter decomposition: a meta-analysis. *Ecology*, **86**, 3252–3257.

Lipson DA, Schmidt SK, Monson RK (1999) Links between microbial population dynamics and nitrogen availability in an alpine ecosystem. *Ecology*, **80**, 1623–1631.

Liski J, Nissinen A, Erhard M, Taskinen O (2003) Climatic effects on litter decomposition from arctic tundra to tropical rainforest. *Global Change Biology*, **9**, 575–584.

McCarthy HR, Oren R, Johnsen KH *et al.* (2010) Re-assessment of plant carbon dynamics at the Duke free-air CO_2 enrichment site: interactions of atmospheric CO_2 with nitrogen and water availability over stand development. *New Phytologist*, **185**, 514–528.

Melillo JM, McGuire AD, Kicklighter DW *et al.* (1993) Global climate-change and terrestrial net primary production. *Nature*, **363**, 234–240.

Melillo JM, Steudler PA, Aber JD *et al.* (2002) Soil warming and carbon-cycle feedbacks to the climate system. *Science*, **298**, 2173–2176.

Nasholm T, Ekblad A, Nordin A, Giesler R, Hogberg M, Hogberg P (1998) Boreal forest plants take up organic nitrogen. *Nature*, **392**, 914–916.

Nasholm T, Kielland K, Ganeteg U (2009) Uptake of organic nitrogen by plants. *New Phytologist*, **182**, 31–48.

Parton WJ, Scurlock JMO, Ojima DS *et al.* (1993) Observations and modeling of biomass and soil organic-matter dynamics for the grassland biome worldwide. *Global Biogeochemical Cycles*, **7**, 785–809.

Raich JW, Rastetter EB, Melillo JM *et al.* (1991) Potential net primary productivity in South America - application of a global model. *Ecological Applications*, **1**, 399–429.

Reiskind JB, Lavoie M, Mack MC (2011) Kinetic studies of proteolytic enzyme activity under varying toluene concentrations. *Soil Biology & Biochemistry*, **43**, 70–77.

Rosenberg MS, Adams DC, Gurevitch J (2000) *MetaWin: Statistical Software for Meta-Analysis*. Sinauer Associates, Sunderland, MA.

Rothstein DE (2009) Soil amino-acid availability across a temperate-forest fertility gradient. *Biogeochemistry*, **92**, 201–215.

Rustad LE, Campbell JL, Marion GM *et al.* (2001) A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. *Oecologia*, **126**, 543–562.

Schimel JP, Bennett J (2004) Nitrogen mineralization: challenges of a changing paradigm. *Ecology*, **85**, 591–602.

Schimel JP, Weintraub MN (2003) The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. *Soil Biology & Biochemistry*, **35**, 549–563.

Schulten HR, Schnitzer M (1998) The chemistry of soil organic nitrogen: a review. *Biology & Fertility of Soils*, **26**, 1–15.

Shaver GR, Canadell J, Chapin FS *et al.* (2000) Global warming and terrestrial ecosystems: a conceptual framework for analysis. *BioScience*, **50**, 871–882.

Thornthwaite C (1948) An approach toward a rational classification of climate. *Geographical Reviews*, **38**, 55–94.

Thornton PE, Rosenbloom NA (2005) Ecosystem model spin-up: estimating steady state conditions in a coupled terrestrial carbon and nitrogen cycle model. *Ecological Modelling*, **189**, 25–48.

Treseder KK (2008) Nitrogen additions and microbial biomass: a meta-analysis of ecosystem studies. *Ecology Letters*, **11**, 1111–1120.

Vitousek PM, Howarth RW (1991) Nitrogen limitation on land and in the sea - how it can occur. *Biogeochemistry*, **13**, 87–115.

Wallenstein MD, McMahon SK, Schimel JP (2009) Seasonal variation in enzyme activities and temperature sensitivities in Arctic tundra soils. *Global Change Biology*, **15**, 1631–1639.

Wang G, Post WM, Mayes MA *et al.* (2012) Parameter estimation for models of ligninolytic and cellulolytic enzyme kinetics. *Soil Biology & Biochemistry*, **48**, 28–38.

Watanabe K, Hayano K (1995) Seasonal-variation of soil protease activities and their relation to proteolytic bacteria and *Bacillus spp.* in paddy field soil. *Soil Biology & Biochemistry*, **27**, 197–203.

Zak DR, Holmes WE, MacDonald NW, Pregitzer KS (1999) Soil temperature, matric potential, and the kinetics of microbial respiration and nitrogen mineralization. *Soil Science Society of America Journal*, **63**, 575–584.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Climate variables for each experimental site.
Table S2. Experimental methods for each site.
Table S3. Weighted regression summary for response of potential proteolytic enzyme activity in the mineral soils to warming and to manipulations that decrease precipitation.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.