Short Communication

Long-term warming alters soil and enzymatic N:P stoichiometry in subarctic tundra

Sari Stark[a,*], Henni Ylänne[a,b], Anne Tolvanen[b,c]

[a] Arctic Centre, University of Lapland, P.O.Box 122, FI-96301 Rovaniemi, Finland
[b] University of Oulu, Department of Ecology and Genetics, P.O. Box 3000, FI-90014 Oulu, Finland
[c] Natural Resource Institute Finland, P.O. Box 413, FI-90014 Oulu, Finland

We found that 19 years of experimental warming in tundra decreased mineral N and increased mineral P pools during mid-growing season. The shift in soil N:P availability paralleled a higher microbial extracellular enzyme activity (EEA) to degrade N-containing organic compounds, a lower EEA to degrade P-containing organic compounds, and lower microbial P. Warming-induced changes in N pools and EEAs interacted with simulated herbivory on deciduous dwarf shrubs; however, the impact of warming on the enzymatic N:P ratio was independent of herbivory. These results indicate that climate warming can alter the stoichiometric nutrient demand of soil microorganisms. Unveiling how warming affects P mineralization and immobilization would substantially improve predictions on tundra ecosystem responses to global change.

Understanding what regulates soil microbial processes in warming Arctic is important as these systems store large quantities of soil organic matter (Davidson and Janssens, 2006). Increasing abundance of deciduous dwarf shrub in response to climate warming (Myers-Smith et al., 2011) may accelerate carbon (C) decomposition through enhanced labile C supply (Buckeridge et al., 2010; Deslippe et al., 2012; Sistla and Schimel, 2013). However, tundra herbivores modify the warming-induced vegetation change (Post and Pedersen, 2008; Väisänen et al., 2014). On warmed nutrient-poor tundra heaths, selective herbivory on deciduous dwarf shrubs may promote higher abundance of unpalatable evergreen dwarf shrubs (Ylänne et al., 2015). As evergreen species are associated with slower soil C and nutrient cycles than deciduous dwarf shrubs (van Wijk et al., 2003), herbivory could partially offset the effects of warming on soil microbial activities.

We tested the above prediction in a 19-year old factorial warming and herbivory experiment in a sub-arctic tundra heath (Kilpisjärvi, 69°30′N, 20°50′E, 670 m asl, long-term mean temperature -2.3 °C). Consistent with Arctic observations, warming by open-top chambers increased deciduous dwarf shrub abundance (Rinnan et al., 2009). However, simulated herbivory (implemented by removing every other current-year shoot from the ramets of the dominant dwarf shrub *V. myrtillus*) had increased the abundance of evergreen dwarf shrub *Empetrum hermaphroditum* especially under warming (Ylänne et al., 2015). Due to increased plant biomass, herbivory had removed more resources under warming (113.6 and 189.3 g C m^{-2} and 2.6 and 3.3 g N m^{-2} in unwarmed and warmed plots, respectively).

We sampled the entire soil organic layer (depth 3.5 cm on average, composite samples of 5–6 soil cores) on three dates in 2013. We analyzed potassium-sulphate extractable soil N and P pools (2 h, 0.5 M K_2SO_4, FIA 5012, Perstorp Analytical, Shimadzu UV-1700) and the total N and P after sieving (2 mm mesh). Microbial N and P were analyzed after chloroform fumigation (Brookes et al., 1985). To depict changes in microbial enzyme production in response to warming (Henry, 2012), six potential extracellular enzyme activities (EEAs) that catalyze organic matter degradation were analyzed using micro-plates with chro-

---

Table 1
The effects warming and simulated herbivory, and their interactions, on soil properties, soil and microbial N and P pools (per g^{-1} SOM), and extracellular enzyme activities (EEAs, μmol h^{-1} g^{-1} SOM) during mid-growing season (July 2013) in a sub-arctic tundra heath after 19 years of field treatments. F- and P-values were obtained by 2-way ANOVA (SPSS Statistics 24), N = 5. Logarithmic transformations (marked with *) were used when necessary to meet the assumptions of ANOVA. Statistical trends ($P < 0.10$) are indicated by italics, and significant effects ($P < 0.05$) by bold.

	Warming		Herbivory		W × H	
	F	P	F	P	F	P
OMC%	0.4	0.548	0.3	0.621	4.4	*0.052*
Moisture%	11.4	**0.004**	0.9	0.347	3.3	*0.087*
pH	1.1	0.312	2.4	0.145	4.0	*0.063*
N pools						
NH$_4$-N*	0.2	0.658	0.0	0.919	5.4	**0.033**
Organic N	1.1	0.319	3.2	0.091	0.2	0.628
Microbial N	0.0	0.849	0.0	0.989	21.0	**< 0.001**
Total N	0.2	0.632	1.6	0.219	2.5	0.136
P pools						
PO$_4$-P*	12.7	**0.003**	7.0	**0.018**	0.9	0.356
Microbial P	13.0	**0.002**	0.1	0.786	0.4	0.542
Total P	2.0	0.176	2.4	0.140	3.4	*0.083*
EEAs						
NAG*	4.0	*0.062*	0.1	0.803	0.3	0.581
LAP	0.6	0.436	0.0	0.874	0.1	0.805
AP	33.9	**< 0.001**	2.6	0.129	4.8	**0.043**
BG	0.6	0.448	1.2	0.299	0.0	0.914
Urease*	3.9	*0.065*	0.1	0.811	5.0	**0.040**
Soil N:P	1.0	0.338	0.2	0.681	0.4	0.542
Microbial N:P	16.9	**< 0.001**	0.7	0.420	6.6	**0.021**
Enzymatic N:P	4.6	**0.048**	0.0	9.901	0.2	0.706

mogenic substrates and urea in acetate buffer (pH 4.4) at room temperature (Sinsabaugh et al., 2000; Martz et al., 2016). We found significant interactions in the treatment effects among the sampling dates (repeated measures ANOVAR; statistics not shown), and consequently tested the data on each date separately (SPSS 22.0 Software).

Most warming effects were limited to mid-growing season (July; Table 1, Figs. 1 and 2) with weak effects during early and late season (Suppl. Tables 1 and 2). During this time, without herbivory, soil NH$_4$-N pool was 37% lower and soil PO$_4$-P pool 124% higher in the warmed than unwarmed plots despite that the total soil N:P ratio remained unchanged (Table 1, W × H interaction in NH$_4$-N; Fig. 1). Simultaneously, warming tended ($P < 0.10$) to enhance the activity of N-acetyl-glucosamidase (52%; NAG, synthesized to derive N through chitin degradation; Sinsabaugh et al., 2008), decreased the activity of acid-phosphatase (20%; AP; synthesized to mineralize P) and microbial biomass P (30%; Table 1, Fig. 2). These findings support a high importance of the stoichiometric nutrient demand (Sinsabaugh and Follstad Shah, 2012) and nutrient availability (Wallenstein et al., 2009; Sistla et al., 2012; Sistla and Schimel, 2013) over EEAs albeit this relationship was evident only in July. These results demonstrated that prolonged climate warming may alter the stoichiometric balance of nutrient-acquiring EEAs (Sistla and Schimel, 2013).

During mid-growing season, when warming effects manifested themselves, plant nutrient uptake is at highest (Schimel and Chapin, 1996) and, owing to plant N uptake and N requirement by soil microorganisms for synthesizing enzymes, tundra systems become increasingly N-limited (Weintraub and Schimel, 2005; Wallenstein et al., 2009). We suggest that the lower soil NH$_4$-N pool in warmed plots indicates this N limitation intensified by warming. Increased NAG activity in response to warming could thus result from enhanced microbial activity for "N mining" through organic matter degradation (Deslippe et al., 2012). Also a high amount of chitin in the soil generally promotes NAG activity (Hernandez and Hobbie, 2010; Zeglin et al., 2013). Yet, warming had no effects on fungal abundance at our site (Rinnan et al., 2009). The effects of warming on P pools were unexpected and difficult to explain. An inverse relationship between PO$_4$-P and EEA for microbial P acquisition agrees with previous findings that free soil P downregulates AP activity (Tibbett et al., 1998), but does not explain why warming decreased microbial P immobilization.

Simulated herbivory interacted with warming but not following our predictions. Noteworthy, herbivory changed how warming affected the N pools, whereas such interactions were not detected on P pools. Under herbivory, warming increased NH$_4$-N (100%) and decreased microbial N (13%, Table 1, significant W × H interactions; Figs. 1 and 2). Herbivory alone decreased soil PO$_4$-P (44%), but did not alter warming effects on PO$_4$-P and microbial P (Table 1, Fig. 1) resulting in a weaker effect on the microbial N:P ratio (Table 1, W × H interaction; Fig. 1). Put together, the changes in N and P pools in response to herbivory support that perturbations affect elemental balances in ecosystems (Sistla and Schimel, 2012). However, as warming decreased urease activity under herbivory and the negative effect of warming on AP activity was even stronger than in the control plots (43%; Table 1, W × H interactions; Fig. 2), changes in EEAs resulted in a same effect of warming on the enzymatic N:P ratio independent of herbivory (Fig. 2). This reveals an astonishing consistency in the enzymatic N:P stoichiometry that was insensitive to the resource loss and the dominant aboveground vegetation.

The opposite effects of warming on N and P, in terms of pools and enzymatic activities, supports a decoupled cycling of these nutrients (Cole and Heil, 1981) but also raises an important question of potential mechanisms behind the observed trends. As microbial P immobilization may largely control subarctic soil PO$_4$-P pools (Jonasson et al., 2004), the lower microbial P in the warmed plots likely explains the higher PO$_4$-P pool. Soil microorganisms immobilize a major proportion of tundra ecosystem P (Giblin et al., 1991; Jonasson et al., 1996) even exceeding the plant P pool (Jonasson et al., 1999), and therefore, even a small decrease in microbial P can easily lead to drastically larger inorganic P pools. The mechanism underlying the decreasing P immobilization in response to warming remains unidentified, but it is known that microbial P immobilization is temporally variable depending on e.g. C availability, temperature and moisture even when the microbial biomass remains stable (Richardson and Simpson, 2011). Importantly, P availability often limits plant growth in tundra (Weintraub, 2011); therefore, if a warming-induced decrease in microbial P represents a common phenomenon, it could exert cascading effects on productivity. Increasing temperatures may also directly decrease AP production in Arctic microbial strains (Tibbett et al., 1998) and alter the temperature acclimation of microbial activity (e.g. Billings and Ballantyne, 2013). Unraveling how warming affects microbial P dynamics could reveal a missing link in understanding indirect mechanisms that govern ecosystem responses to global change.