Dear Dr. van Bodegom,

thank you for your positive evaluation of our manuscript. We incorporated the raised points and now formulate more carefully regarding the point of N uptake. Parts of the method and discussion section were changed. We don’t talk about N incorporation anymore and now state in the discussion: “Lower or stable above-ground biomass and lower AT% 15N values combined are a clear hint for reduced N uptake by the affected plant species.” We stayed with AT% values, since a total N mass balance is not possible with our data. The information regarding decreasing and stable above-ground biomass is now included more prominent and also included in figure caption 4. Plant available N data was transformed to µg N cm^{-2} d^{-1} as is figure 2.

Best regards

Jan Schürings and co-authors

Increased winter soil temperature variability enhances nitrogen cycling and soil biotic activity in temperate heathland and grassland mesocosms

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ABSTRACT

Winter air temperatures are projected to increase in the temperate zone, whereas snow cover is projected to decrease, leading to increased soil temperature variability, and potentially to changes in nutrient cycling. Here, we experimentally evaluated the effects of increased winter soil temperature variability on selected aspects of the N-cycle in mesocosms containing different plant community compositions. The experiment was replicated at two sites, a colder mountainous upland site with high snow accumulation and a warmer and dryer lowland site.

Increased soil temperature variability enhanced soil biotic activity for both sites during winter, as indicated by 35% higher nitrogen (N) availability in the soil solution, 40% higher belowground decomposition and a 25% increase in the potential activity of the enzyme cellobiohydrolase. The mobilization of N differed between sites, and the ^{15}N signal in leaves was reduced by 31% in response to winter warming pulses, but only at the cold site, with significant reductions occurring for three of four tested plant species at this site. Furthermore, there was a trend of increased N leaching in response to the recurrent winter warming pulses.

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Overall, projected winter climate change in the temperate zone, with less snow and more variable soil temperatures, appears important for shifts in ecosystem functioning (i.e. nutrient cycling). While the effects of warming pulses on plant N mobilization did not differ among sites, reduced plant ^{15}N incorporation at the colder temperate site suggests that frost damage may reduce plant N uptake in a warmer world, with important implications for nitrogen cycling and nitrogen losses from ecosystems.

1 Introduction

Winter soil temperature is an important driver for many ecological and biogeochemical processes in the cold-temperate and boreal zone, and it can influence the activity of plants and soil biota (Matzner and Borken, 2008; Kreyling, 2010). While microbial activity and nitrogen (N) cycling continue below freezing (Clein and Schimel, 1995; Mikan et al., 2002), higher mean soil temperatures are generally expected to cause exponentially higher soil biotic activity (Rustad et al., 2001; Melillo et al., 2002). Consequently, winter warming can result in increased N mineralization and N availability in the soil solution in the following growing season (Turner and Henry, 2010). Warmer soils over winter increase soil biotic activity, e.g. soil respiration, decomposition by soil fauna and microbes, higher enzymatic activity, higher N mineralization, etc. This holds true especially towards the end of winter, and can accelerate plant productivity (Schuerings et al., 2013). Since plants are capable of winter N uptake (Grogan et al., 2004; Andresen and Michelsen, 2005), their activity could counteract N leaching (Patil et al., 2010). The general effectiveness of plants in taking up N over winter, however, is not fully clear until now. Comparable N uptake rates over winter and summer have been reported for some species (Nasholm et al., 2000; Bardgett et al., 2003), but there is also evidence that cold acclimation reduces the potential for N uptake (Malyshev and Henry, 2012a).

Due to increased winter air temperatures, snow cover will decrease in many regions of the temperate zone (Christensen et al., 2007; Kreyling and Henry, 2011). However, air frost events will still occur with unchanged magnitude and duration as nowadays in many temperate regions (Kodra et al., 2011), and with less insulating snow cover, winter soil temperatures can become more variable, particularly in upland and cold temperate regions (Henry, 2008; Brown and DeGaetano, 2011). The resulting more variable soil temperature conditions with frequent soil frost and freeze-thaw cycles (FTC) can affect N cycling. Soil frost and FTC can physically damage plant roots (Tierney et al., 2001) and therefore reduce the plants ability to take up N (Campbell et al., 2014), break up soil aggregates (Oztas and Fayetorbay, 2003), and lyse microbial cells what enlarges the easily available N pool (Skogland et al., 1988), thereby affecting N cycling and leading to N losses in dissolved (Boutin and Robitaille, 1995; Brooks et al., 1998; Joseph and Henry, 2008) or gaseous forms (Matzner and Borken, 2008). For warmer, lowland temperate regions, however, although soil temperature variability might still increase (Kreyling, 2010), an increase in winter air temperatures could lead to fewer soil FTC due to less frost (e.g. lowland Germany, Kreyling and Henry, 2011). Contrasting effects of winter climate change can therefore be expected for colder (stronger effects due to greater increase in soil temperature variability) versus warmer (naturally higher soil temperature variability) temperate regions, and studies of biogeochemical responses to increased soil temperature variability should be designed to account for these differences.

Finally, plant species and vegetation types are known to influence N cycling (Hooper and Vitousek, 1998; Knops et al., 2002). Different plant species and communities further show different reactions to increased winter temperature variability in the temperate zone, with grasses appearing more responsive than dwarf shrubs (Kreyling et al., 2010; Schuerings et al., 2014) regarding their productivity, probably due to their faster life-cycle. However, this increased responsiveness in productivity of grasses can either be beneficial (Kreyling et al., 2008), or detrimental (Schuerings et al., 2014), probably depending on whether the minimum temperatures experienced after warm phases induce frost damage. Altered plant productivity can therefore indirectly affect N cycling. Generally, stress resistance is linked to nitrogen or nutrient stress tolerance (Macgillivray et al., 1995). Moreover, increased N availability over winter can increase the risk of frost damage to plants (Malyshev and Henry, 2012b).

In this experiment we tested the effects of more variable winter temperature conditions, i.e. recurrent, short winter warming pulses, on soil biotic and potential extracellular enzyme activity, N availability in the soil solution, and N uptake by plants in different plant communities (grassland, heathland; same communities as in Schuerings et al., 2014) at two sites with contrasting winter climate (a warm, snow-poor lowland and a cold, snow-rich upland site). We hypothesised that (1) recurrent winter warming pulses would enhance N-cycling (i.e. increased N availability, soil biotic activity and N uptake into plants). (2) We further expected different responsiveness to the recurrent warming pulses at the two sites, with more variable soil temperatures and stronger frost, therefore frost damage negatively affecting plant N uptake at the colder upland site. (3) Finally, we expected differences among the plant communities in the response of N cycling to the recurrent warming pulses, with a higher ability for winter N uptake in grassland than in heathland plants.

2 METHODS

2.1 Experimental design and site description

This research is part of the EVENT IV experiment, testing the effects of increased winter temperature variability on temperate heath and grassland communities. The effects of the recurrent warming pulses on plant growth (above- and below-ground) are summarized in Schuerings et al. (2014), whereas here we concentrate on nitrogen cycling. The experiment was replicated at two sites: the warm site was located in the Ecological-Botanical Garden of the University of Bayreuth (49° 55' 36.32'' N, 11° 34' 57.28'' E, 358 m a.s.l.) and the cold site was located at the Waldstein mountain in the Fichtelgebirge (50° 8' 35.81'' N, 11° 51' 50.92'' E, 781 m a.s.l.). The cold site generally experiences more precipitation and harsher winter conditions (Table 1).

The experiment consisted of three fully crossed factors: (1) increased winter temperature variability by application of winter warming pulses versus ambient reference conditions, (2) two experimental sites with naturally different winter climate, (3) six different plant communities and an additional bare ground control. The plant communities consisted of three grassland communities (monocultures of the grass *Holcus lanatus* (L.) and the herb *Plantago lanceolata* (L.), and a community with a mix of both species) and three heathland communities (monocultures of the dwarf shrub *Calluna vulgaris* (L.) and the grass *Deschampsia flexuosa* (L.) and a community with a mix of both species). All species present in this experiment are very common perennial species in Central Europe. In addition, there was a bare ground control in every block. Plant communities were blocked and randomly assigned to the winter warming pulses manipulation and ambient reference. Temperature manipulation blocks, and therefore each factorial combination, were replicated five times. This setup was fully replicated at both experimental sites. For the 140 plots, plastic barrels with 0.2 m^2 surface (50 cm diameter) and 80 cm depth were used as mesocosms. Each of the six mesocosms per treatment was placed in a corner of a hexagon, with 30 cm distance between mesocosms and at least 50 cm separation from the hexagon edge. The bare ground control was placed in the middle of the hexagons. All space between the mesocosms was filled with the same substrate as used within the mescosms. The soil substrate was homogenized loamy sand (77% sand, 16% silt, 7% clay) from a nearby sand quarry (where all used plant species naturally occur), with a pH=7.35 (measured in 1 M KCl) and a total carbon content of 2.37%. The barrels were attached with outlet hoses at the bottom of each mesocosm, so that the mesocosms functioned as zero tension lysimeters. Sixteen plants per mesocosm were planted in a systematic grid in May 2010. All plants were grown from seed in January 2010, except for the dwarf-shrub *C. vulgaris*, which was obtained as 2-year old individuals in February 2010.

2.2 Manipulation of winter temperature variability

Winter warming pulses were applied with six IR-heating lamps (250 W) located in between the mesocosms at a height of 60 cm and surface heating wires (distance 20 cm, 400 W per block), which resulted in 1900 W per block (7 mesocosms). The ambient reference mesocosms were equipped with dummy lamps. Six warming pulses were administered simultaneously for both sites between 15 December 2010 and 28 February 2011 (see Fig. 1). Warming pulses were administered when there was soil frost at both sites and weather forecast predicted further air frost for at least the next 48 h.

Soil temperature (-2 cm; once in every treatment and reference block; 10 measurements per site and 20 in total) and air temperature (+5 cm; one treatment and reference block per site; 2 measurements per site and 4 in total) were measured hourly by thermistors (B57863-S302-F40, EPCOS AG, Germany) connected to a datalogger (dl2, Delta-T Devices Ltd, UK). To quantify the effect of the warming pulses treatment on soil temperature variability, we calculated the coefficient of variation (CV = standard deviation x hourly mean^{-1} x 100; temperatures were converted to K for this). Snow height was measured each morning via a webcam picture of a measuring stick.

2.3 Response parameters

Plant available N was measured via the resin stick method (Plant-root-simulator (PRS^{TM})-probes; Western Ag Innovations Inc., Canada). Two cation and two anion PRS^{TM}-probes were installed vertically with a distance of 20 cm to each other (0 -15 cm depth) per mesocosm prior to the warming pulse manipulation on 18 December 2010 and collected on 17 March 2011 after the winter warming pulses treatment. PRS^{TM}-probes were cleaned and kept in a fridge until being sent to Western Ag Innovations Inc. (Canada) in a cool box for analysis. For the statistical analysis, nitrate and ammonium were pooled due to low ammonium concentrations. The maximum ion capacity of the probes for nitrate is 2088 µg 10 cm^{-2}. The values in our study are far lower, showing that the system was not saturated. For better comparability to other studies we give mean plant available N per cm^{-2} and day. But it is important to note that N uptake by resin sticks is not a linear process.

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Soil biotic activity, i.e. decomposition by microorganisms and feeding by soil fauna, was measured via bait-lamina sticks (terra protecta GmbH, Germany) (Kratz, 1998). One bait-lamina stick containing 16 baits was inserted vertically in the top soil layer of every mesocosm prior to the warming pulses treatment on 18 December. The baits consisted of a mixture of powdered cellulose, bran flakes and active coal. These baits are potentially eaten by earthworms, macro- to micro arthropods and additionally are decomposed by soil microorganisms. The sticks were collected after the winter warming pulses treatment on 17 March, cleaned, and the number of eaten baits was counted. For the latter, sticks were placed on a light bench and when light shined through the baits they were counted as eaten. This analysis was done by a single person who was blind to the factors.

For the potential extracellular enzymatic activity (PEEA), which we used as another proxy for soil biotic activity and decomposition, three soil samples (2 cm diameter, 10 cm depth) per mesocosm were collected and mixed for assays of potential extracellular enzyme activity in soil on 21 February 2011. Soil samples were stored in airtight plastic zip-bags at 4°C and were analysed within 3 days. PEEA assays were carried out with Methylumbelliferone substrates (MUF) (Pritsch et al., 2004; Pritsch et al., 2005). The following PEEAs were measured: MU-β-D-glucopyranoside (MU-G), for β-glucosidase, MU-β-cellobioside (MU-C) for cellobiohydrolase, MU-β-D-xylopyranoside (MU-X) for xylosidase, MU-phosphate (MU-P) for acid phosphatase. Substrates and calibration saturation and incubation times were determined in pre-experiments (data not shown) as follows: MU-G and MU-X each 500 µM incubating for 60 min, MU-C 500 µM incubating for 120 min, MU-P 800 µM incubating for 40 min. Fluorescence was detected at an excitation wavelength of 360 nm and an emission wavelength of 450 nm with a Gemini EM Fluorescence Microplate Reader from Molecular Device, California.

Prior to the warming pulses treatment (18 December 2010), plots were labelled with 0.02 g Potassium Nitrate-^{15}N (min. 99.19 atom % 15N; Campro Scientific GmbH, Germany), dissolved in 250 ml deionized water, resulting in 0.1 g ^{15}N m^{-2}. Leaf (2-3 medium aged leaves per plot and species, randomly chosen), root (fine roots from a soil sample taken directly next to a randomly chosen plant per mesocosm and species) and soil samples (3 soil samples per plot were mixed; 2 cm diameter, 10 cm depth) were taken on 17 March 2011, after the winter warming pulses treatment. The samples were kept frozen until they were cleaned, dried (48 h at 50° C) and ball milled. Mass spectroscopy analysis was done at the laboratory of Isotope Biogeochemistry, BayCEER, University of Bayreuth, with a combination of an elemental analyzer (Carlo Erba NC 2500, CE Instruments, Italy) and an isotope mass spectrometer (delta plus, Thermo Fisher Scientific, Germany). Atom % increase values for plant and soil material collected after the winter warming pulses treatment were calculated by comparing to values obtained from unlabelled reference plants (n = 5 per species) and soil material taken prior to the winter warming pulses treatment (n = 3 per experimental site). Due to missing volume readings, the isotopic signature of leachate could only be determined and related to volume of leachate for four mesocosms (*Holcus lanatus* and *Plantago lanceolata* mixed mesocosms at both sites for both winter warming pulses treatments), which were permanently equipped by tipping buckets (7041.3000X, Theodor Friedrichs & Co., Germany). Therefore, no mass balancing of the label was possible, and we report ^{15}N-atom% here. For interpretation of the data it is important to note that overall above-ground biomass significantly decreased by 9.2 % due to the warming pulses treatment (Schuerings et al., 2014). For single species, only *H. lanatus* showed a strong decrease by 29.2 % whereas the other species showed no significant treatment effects (Schuerings et al., 2014).

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2.4 Data analyses

Linear mixed-effect models combined with analysis of variance (ANOVA) were applied to test for significant winter warming pulses treatment, site and plant community effects. All possible interactions of community or species and site with the warming pulses treatment were included as fixed effects (s. Table 2 & 3 for all tested interactions). For the analysis of ^{15}N content in plants, species identity was included as a fixed factor instead of community composition, whereas community was included as a random effect. Block identity was set as a random effect in all models, thereby accounting for the blocked design. Before statistical analysis, we tested for normality and homogeneity of variance by examining the residuals versus fitted plots and the normal qq-plots of the linear models (Faraway, 2005). If conditions were not satisfactorily met, we applied log(x)- (plant available N; ^{15}N atom% increase of leaves and roots; PEEA of beta-glucosidase, cellobiohydrolase, xylosidase), log(x+1)- (^{15}N atom% increase in soil), or sqrt(x)- (PEEA acid phosphatase) transformation. Significance level was set to $p < 0.05$. All statistical analyses were performed using R 2.12.2 (R Development Core Team 2011) and additional packages nlme (Version 3.1-98, 2011) and sciplot (Version 1.0-9, 2011) for graphical illustrations.

3 RESULTS

The winter warming pulses manipulation successfully decreased snow cover and resulted in increased soil temperature variability (Fig. 1). At the warm site, variation in soil temperature during the manipulation period (15 December 2010 to 28 February 2011) was increased to CV = 0.99 in comparison to CV = 0.66 in the reference mesocosms. Mean soil temperature increased to 1.8°C in the manipulation as compared to 0.1°C in the ambient reference. Minimum temperature reached -4.2 °C and -4.0 °C, respectively. For the cold site, variation in soil temperature during the manipulation period increased to CV= 0.68 in comparison to CV= 0.43 in the reference mesocosms. Mean soil temperature was almost unchanged with -0.1°C in the warming pulses manipulation and -0.3°C under ambient reference conditions. However, minimum temperature was considerably lower in the warming pulses mesocosms, reaching -4.7 °C, as compared to -2.6 °C in the reference mesocosms. The number of soil freeze thaw cycles was not altered noticeably at any site (warm site: 7 vs. 8, cold site: 6 vs. 5).

Plant available nitrate and ammonium significantly increased by 34.5% in response to the winter warming pulses treatment (F=13.5, p<0.001; Table 2, Fig. 2). The cold site overall had a 48.4% higher amount of N available than the warm site (F=20.0, p<0.001; Table 2, Fig. 2). Plant community composition also influenced plant available N (F=18.4, p<0.001; Table 2, Fig. 2). Bare ground control mesocosms had the highest N values, followed by the heathland communities and then the grassland communities, with only monocultures of *H. lanatus* reaching levels of the heathland communities. Winter warming pulse effects were not influenced by site or plant community (no significant interactions, Table 2).

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Soil biotic activity, i.e. the number of eaten baits, increased by 40% (F=17.5, p<0.001; Table 2, Fig. 2) due to the winter warming pulses treatment in comparison to reference conditions. Soil biotic activity did not significantly differ between sites or plant communities. The warming pulses effect, however, was influenced by the plant communities (F=2.3, p=0.037), with slightly decreasing activities in monocultures of *P. lanceolata* and mixed communities of *C.*

vulgaris & *D. flexuosa* due to the warming pulses (Fig. 2). All other communities showed an increase in soil biotic activity due to the warming pulses. No other interaction with the warming pulses treatment yielded significance for soil biotic activity (Table 2).

Regarding PEEA there was a general trend towards higher values under the winter warming pulses treatment, yet only for cellobiohydrolase was this effect statistically significant (F=5.3, p=0.035). For the other three tested enzymes no significant effect of the winter warming pulses treatment was observed. Generally, there were significantly higher PEEAs at the cold site than at the warm site (Table 2, Fig. 3) and plant community composition effects differed such that, except for acid phosphatase, grassland communities showed higher PEEA than heathland communities (Table 2, Fig. 3). No significant interactions between the warming pulses treatment and site or plant community were observed (Table 2).

The AT% ^{15}N values in leaves were significantly reduced by 21.7% (relative difference) under the winter warming pulses treatment in comparison to reference conditions (F=5.9, p=0.016), whereas for root and soil material no significant winter warming pulse effect was observed (Table 3, Fig. 4). For leachate, no statistical analysis was performed due to the low replication, but for the existing samples (n=2 per winter warming pulses treatment), a clear trend towards increased leaching of the ^{15}N-tracer was observed (Fig. 4). Generally, the cold site showed significantly higher plant AT% ^{15}N values than the warm site (Table 3, Fig. 4). *D. flexuosa* exhibited the highest AT% ^{15}N values, followed by *P. lanceolata*, with the same pattern observed for leaves and roots. Significant decreases in the ^{15}N signal in plant leaves (-30.7%) in response to warming pulses only occurred at the cold site (winter warming pulses treatment x site interaction: F=8.6, p=0.004; Table 3, Fig. 4). The significant three-way interaction between warming pulses treatment, site, and species identity (F=3.4, p=0.004) indicated that the decrease in ^{15}N values only happened at the cold site and only for three of the four species (*C. vulgaris*, *D. flexuosa* and *H. lanatus*; Fig. 4).

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4 DISCUSSION

Recurrent winter warming pulses led to increased soil temperature variability and influenced N cycling in our experiment. As expected, N availability was increased (+35%) in the mesocosms which received the winter warming pulses treatment. Increased N availability during winter/early spring is often explained by freeze-thaw events resulting in increased biological and physical decomposition of soil organic matter (SOM) (Matzner and Borken, 2008) and increased N mineralization (Rustad et al., 2001; Melillo et al., 2002). Yet, in our study FTC frequency was merely changed between winter warming pulses and references plots (±1), implying that the warming pulses treatment affected N availability either through increased temperature variability or the increase in mean temperature. Due to the winter warming pulses soil biotic activity increased by 40%. This increase in soil biotic activity is in line with results from other winter warming experiments which measured soil respiration as an index of soil biotic activity (Davidson and Janssens, 2006; Allison and Treseder, 2011). The soil enzymes we examined play a major role in the decomposition of biological material (Marx et al., 2001). We observed significantly increased PEEA for cellobiohydrolase, whereas for the other three tested enzymes the observed increases were not significant. Therefore in our experiment, increased soil temperature variability led to increased biotic decomposition as indicated by increased soil biotic activity and increased PEEA of cellobiohydrolase. In winter warming experiments, increased N cycling is often attributed to changes in the frequency of soil FTC (Mikan et al., 2002). Despite only small changes in FTC frequency in our mesocosms, however, we observed increased N availability, increased soil biotic and soil potential enzymatic activity. However, for the cold site, where it is important to note that mean soil temperature only increased by 0.2 °C, mean minimum temperature was considerably lower in the warming pulses mesocosms, reaching -4.7 °C, as compared to -2.6 °C. Since we found lowered N incorporation into plants (see discussion further down) and stable or lower plant biomass (Schuerings et al., 2014) at the cold site, this could have lowered N immobilization by plants. The temporal dynamics of soil temperature, in particular the intensity of freezing right after warming pulses, is therefore another important determinant of N cycling responses, possibly leading to frost damaging of dehardend plants. While changed FTCs (Joseph and Henry, 2008), warmer mean soil temperatures (Rustad et al., 2001; Melillo et al., 2002) and single extreme frost events (Elliott and Henry, 2009) are known to be important drivers of N cycling, our results imply that soil temperature variability, i.e. temperature dynamics, can also affect N availability and soil biotic activity.

We found significantly higher N availability and potential activity of all four tested potential soil enzymes for the cold site despite lower mean temperatures at the site. Groffman et al. (2009) found the same pattern along an altitudinal gradient in a northern hardwood forest. This suggests that the local climate may have an important influence on the magnitude of N mobilization processes. However, since we found no significant interaction between winter warming pulses treatment and site, the effects of winter warming pulses on N availability, soil biotic activity and potential soil enzymatic activity therefore appear independent of the local climate.

The mobilization of N was influenced by the plant community composition, with the bare ground control showing highest levels of available N. Since there were no roots in the bare ground plots competing with the PRS^{TM}-probes for N, this result is not surprising. Regarding plant communities, there was no clear pattern in N availability, although the heathland communities showed higher values than grassland communities with the exception of monocultures of *H. lanatus*, which showed similar values as the heathland communities. The interaction between the warming pulses treatment and plant community indicated that plant species composition influenced soil biotic activity differently under winter warming pulses. However, there was no clear pattern, since all communities showed increased soil biotic activity in response to the winter warming pulses, except for monocultures of *P. lanceolata* and mixed cultures of *C. vulgaris* and *D. flexuosa*. Potential soil enzymatic activity was generally higher in grassland mesocosms in comparison to heathland mesocosms, with the exception of acid phosphatase.

The ^{15}N signal in plants leaves was, contrary to our expectations, decreased by the winter warming pulses treatment. Plants can lose their cold hardiness within hours in response to elevated temperatures (Kalberer et al., 2006), and subsequent frost events after a winter warm spell can thus damage plants substantially (Bokhorst et al., 2009). Freezing intensity is also an important determinant of plant frost damage, and while most temperate species can tolerate temperatures at or below freezing, there is often a threshold subfreezing temperature where damage intensifies (Malyshev and Henry, 2012a). Notably, the minimum temperatures reached in the reference mesocosms at the cold site were the least severe, and the highest

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AT% ^{15}N values were observed in these plots, whereas minimum soil temperatures of at least -4 °C were reached in the treatment plots at the cold site and in all of the warm site mesocosms, all of which featured relatively low ^{15}N values. Similarly, in other systems, grass ecotypes located at northern sites that are protected from cold air by thick snow cover have developed lower frost tolerance than conspecific ecotypes located in warmer locations that feature less snow cover, because the latter ecotypes experience more intense frost (Dionne et al., 2010).

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We also observed significant differences among the tested species in the increase of AT% ^{15}N values, which is not surprising, given that species exhibit wide variation in their nutrient uptake capacities (Hooper and Vitousek, 1998; Knops et al., 2002). The interesting point is that the reduction in ^{15}N values only happened at the cold site and only for *C. vulgaris*, *D. flexuosa* and *H. lanatus* (interaction: winter warming pulses treatment x site x species). Total above-ground biomass of all tested species decreased by 9.2 % in response to the winter warming pulses treatment (Schuerings et al., 2014), thus dilution effects on N-tracer uptake can be excluded. Lower or stable above-ground biomass and lower AT% ^{15}N values combined are a clear hint for reduced N uptake by the affected plant species. Such differences among species in frost susceptibility could have important consequences for competitive balances and shifts in community composition over the long term (Joseph and Henry, 2008; Cornelissen and Makoto, 2014).

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Chronic winter warming can increase above-ground biomass (Hutchison and Henry, 2010; Natali et al., 2012; Schuerings et al., 2013). This additional growth may be fuelled by increased N mobilization in early spring. Pulsed winter warming increasing the risk of frost damage, however, complicates this simple expectation of increased plant growth under winter climate change. The inability of frost-damaged plants to take up the available N in the soil solution might trigger N losses from ecosystems by N leaching or gaseous losses (Ineson et al., 1998; Campbell et al., 2014). In this experiment we also found species-specific responses in above-ground biomass production due to the winter warming pulses (Schuerings et al., 2014); only *H. lanatus* showed a decrease in above-ground biomass, whereas the other tested species remained unaffected by the winter warming pulses treatment in their above-ground productivity. Taken together, species- or vegetation type-specific responses have to be taken into account when forecasting effects of climate change on N-cycling (Makoto et al., 2014). Furthermore, regarding winter climate change, pulsed warming events can result in opposing effects on N cycling and biomass accumulation than chronic warming.

5 CONCLUSIONS

Future winters in the temperate zone are expected to be characterized by more variable soil temperatures due to increasing air temperature variability and due to missing insulation by snow. Our experiment implies that more variable soil temperatures enhance nitrogen mobilization in the soil independent from vegetation types and the local climate. Plant performance, however, depended on local climate, with plant ^{15}N immobilization during winter and early spring after exposure to winter warming pulses being reduced at colder sites, probably due to frost damage after the warming pulses. This pattern implies increased risk for nitrogen leaching at colder temperate sites in response to increased winter temperature variability. Taken together, our findings emphasize the importance of temperature variability, plant performance, and frost damage in a warmer world for nitrogen cycling and nitrogen losses from ecosystems.

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TABLES

Table 1: Climate characteristics of the two experimental sites, measured on site by the department of Micrometeorology until 2008; University of Bayreuth, Prof. T. Foken (Schuerings et al., 2014)

Parameter (Unit; start of measurements warm site / cold site)	**Warm site**	**Cold site**
Mean annual temperature (°C; 1998 / 1994)	8.8	5.0
Mean winter temperature (DJF; °C; 1998 / 1994)	0.6	-2.0
Mean annual precipitation (mm; 1998 /1994)	717	1002
Mean winter precipitation (DJF; mm; 1998 / 1994)	158	237
Mean # of days with soil frost (-5 cm) (2003 / 1999)	19	31

Table 2: ANOVA-results of all tested main and interaction effects for N mobilization, i.e. N availability in the soil solution (NH_4^+ and NO_3^-), soil biotic activity (bait-lamina test), and the four tested potential soil enzyme activities. Warming pulses: Winter warming pulses treatment.

Factor	**N availability in soil solution**		**Soil biotic activity**		**Beta-glucosidase activity**		**Cellobiohydrolase activity**		**Acid phosphatase activity**		**Xylosidase activity**	
	F	P	F	P	F	P	F	P	F	P	F	P
Warming pulses	**13.5**	**<0.001**	**17.5**	**<0.001**	1.8	0.199	**5.3**	**0.035**	2.6	0.127	2.0	0.173
Site	**20.0**	**<0.001**	0.6	0.441	**67.2**	**<0.001**	**69.2**	**<0.001**	**12.6**	**0.003**	**33.6**	**<0.001**
Community	**18.4**	**<0.001**	0.3	0.912	**23.5**	**<0.001**	**16.2**	**<0.001**	**32.5**	**<0.001**	**44.5**	**<0.001**
Warming pulses x Site	0.6	0.425	0.9	0.358	3.2	0.094	1.3	0.266	0.9	0.359	3.8	0.068
Warming pulses x Community	0.2	0.961	**2.3**	**0.037**	1.4	0.213	0.7	0.663	1.1	0.388	0.6	0.694
Warming pulses x Site x Community	0.6	0.715	1.1	0.370	0.7	0.685	1.0	0.400	0.9	0.500	1.4	0.212

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Gelöscht: 2

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Gelöscht: 415

Gelöscht: 3

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Gelöscht: 9

Gelöscht: 547

Table 3: ANOVA-results of all tested main and interaction effects for the fate of a ^{15}N label (increase in atom % ^{15}N in the compartments leaves, fine roots, and bulk soil). Warming pulses: Winter warming pulses treatment.

	^{15}N atom % increase					
Factor	**Leaves**		**Roots**		**Bulk soil**	
	F	P	F	P	F	P
Warming pulses	**5.9**	**0.016**	1.5	0.228	0.9	0.331
Site	**144.5**	**<0.001**	**19.3**	**<0.001**	**29.9**	**<0.001**
Species/Community (Soil)	**7.4**	**<0.001**	**9.6**	**<0.001**	1.7	0.134
Warming pulses x Site	**8.6**	**0.004**	2.1	0.153	2.0	0.162
Warming pulses x Species	1.2	0.313	0.5	0.695	0.7	0.647
Warming pulses x Site x Species	**3.4**	**0.004**	1.0	0.422	1.2	0.292

FIGURES

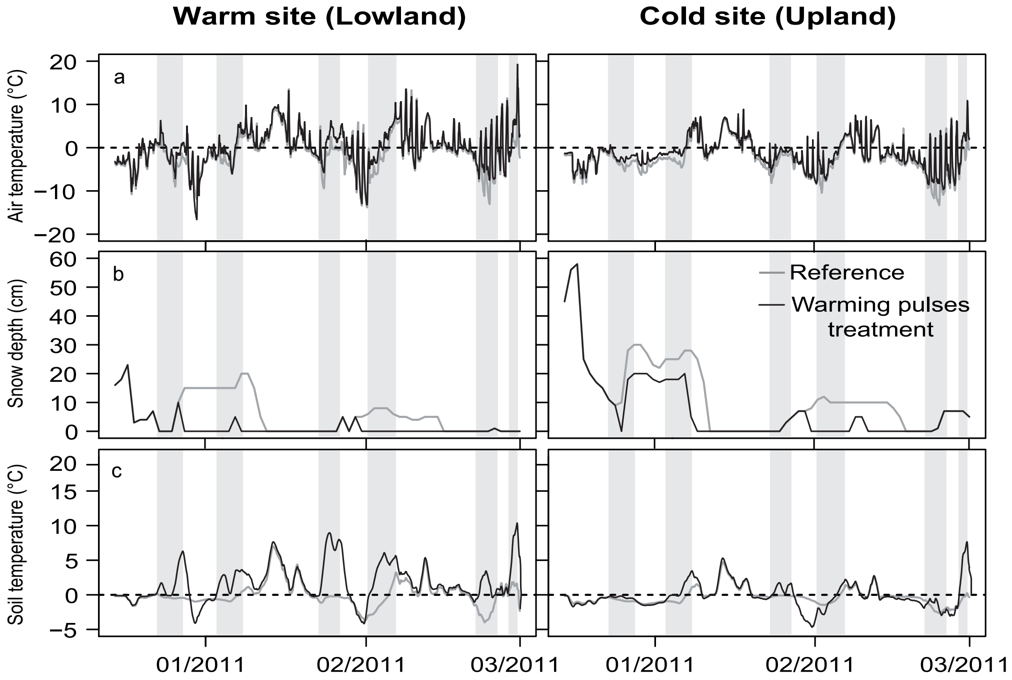

Fig. 1 Mean daily air temperature at +5 cm (a), snow depth (b) and mean daily soil temperature at -2 cm (c) at the two experimental sites for the winter warming pulses treatment (black line) and reference conditions (grey line). Warming pulses (grey boxes) were applied between 15th December 2010 and 28th February 2011 (Schuerings et al., 2014).

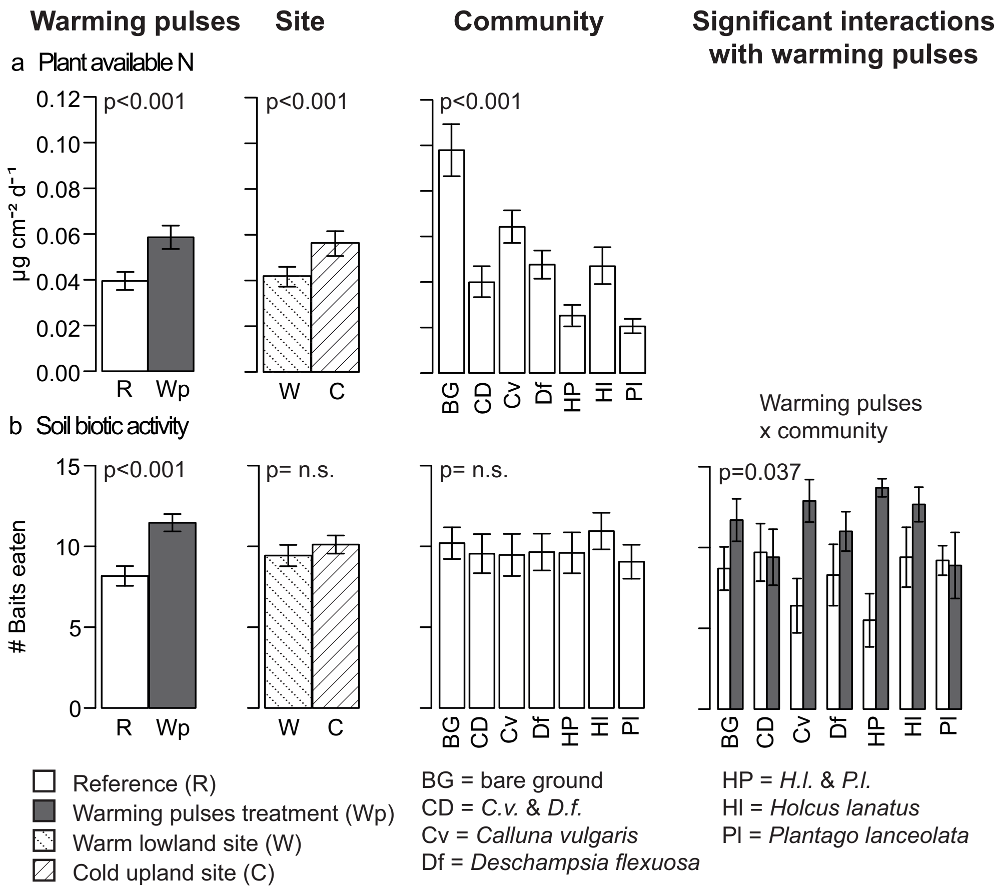

Fig. 2 (a) Plant available nitrogen (nitrate and ammonium; PRS^{TM}-probes) and (b) soil biotic activity (bait-lamina test) during the manipulation period (18 December 2010 - 17 March 2011). Main winter warming pulses treatment, site and community effects and all significant interactions between the winter warming pulses treatment with site and community are shown. Mean (± S.E.) values are shown (n=140).

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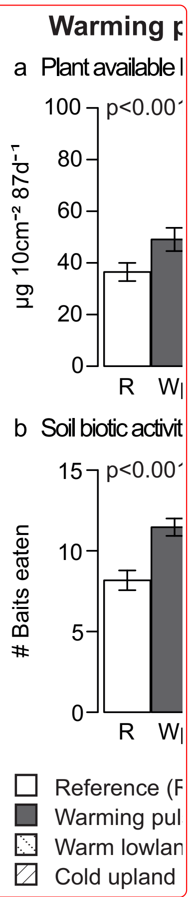

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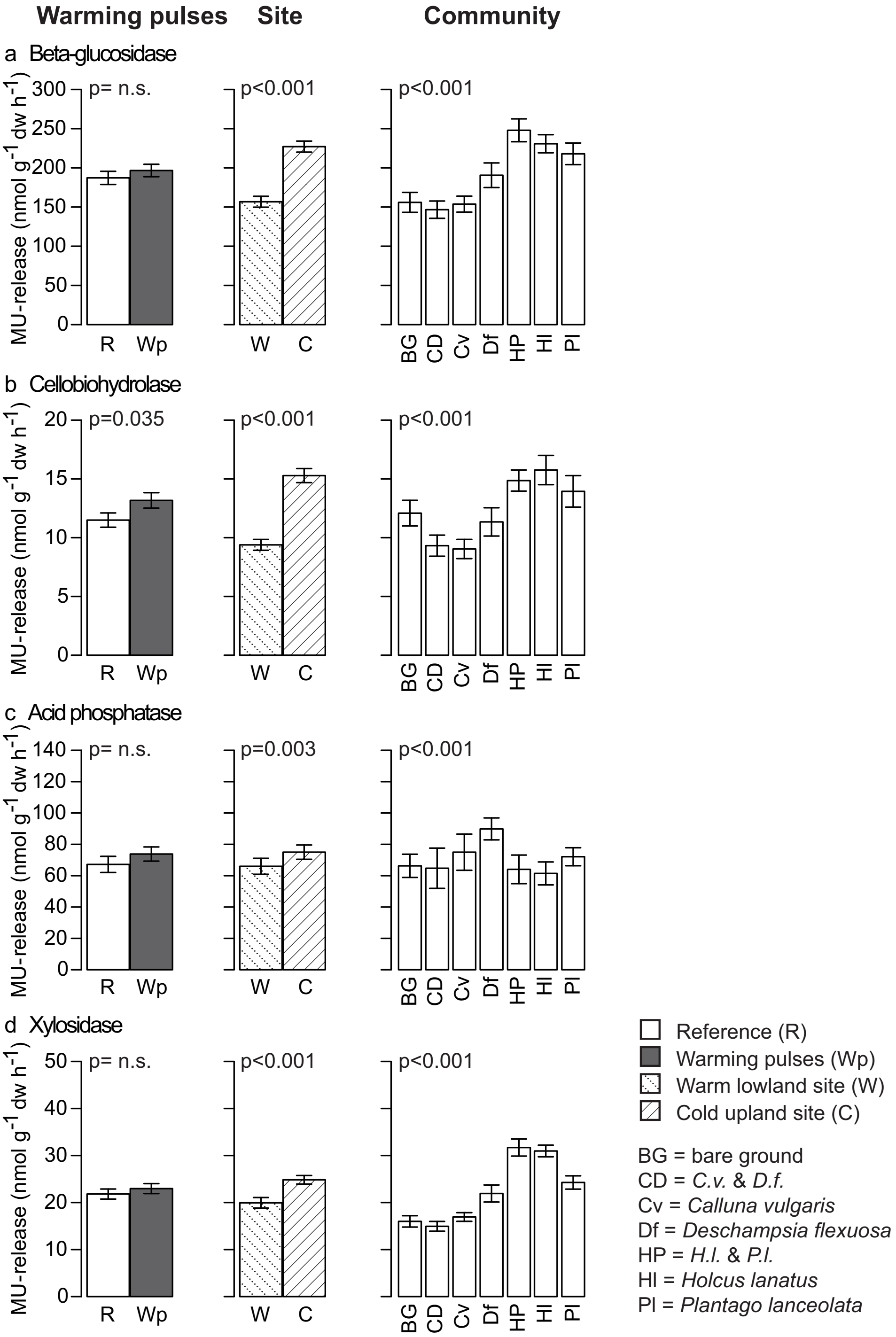

Fig. 3 Mean potential soil enzymatic activity for the four tested enzymes (a) β-glucosidase, (b) cellobiohydrolase, (c) acid phosphatase and (d) xylosidase (all ± S.E.) during the manipulation period (18 December 2010 - 17 March 2011). Main winter warming pulses

treatment, site and community effects are shown. No significant interactions between the winter warming pulses treatment with site and community were detected.

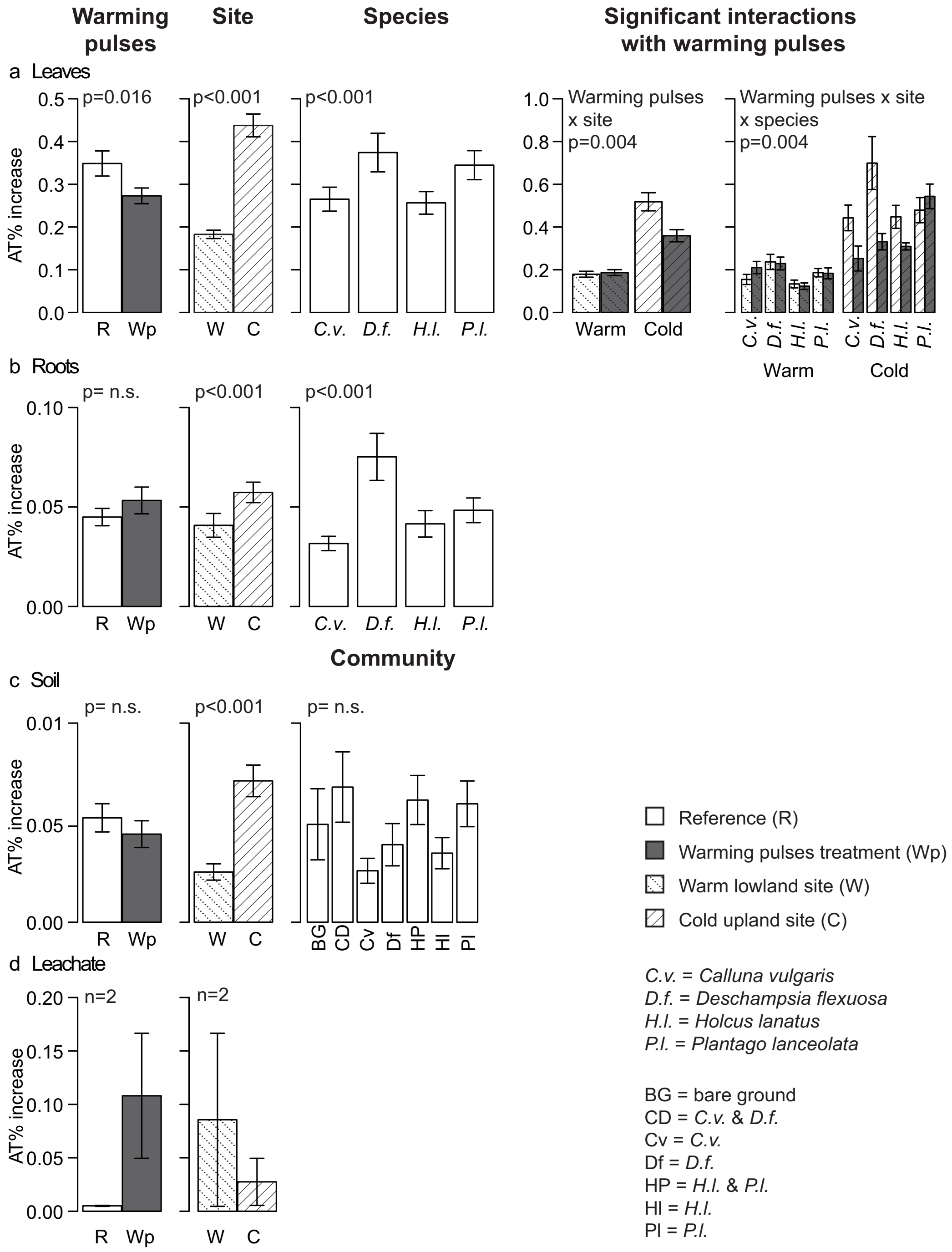

Fig. 4 Mean increase in atom% values (± S.E.) for leaves (n=80), roots (n=80), bulk soil (n=70) and leachate (n=2). Before the warming pulses treatment all plots were watered with 0.25 l of water with 0.02 g Potassium Nitrate-^{15}N (min. 99.19 atom % ^{15}N). Main winter warming pulses treatment, site and community effects and all significant interactions between the winter warming pulses treatment with site and community are shown. It is

important to note that total above-ground biomass declined by 9.2 % in the growing season after manipulations, so that tracer dilution effects due to increasing biomass can be excluded (Schuerings et al., 2014).