A meta-analysis of experimental warming effects on terrestrial nitrogen pools and dynamics

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Summary

- Global warming may have profound effects on terrestrial ecosystems. However, a comprehensive evaluation of the effects of warming on ecosystem nitrogen (N) pools and dynamics is not available.
- Here, we compiled data of 528 observations from 51 papers and carried out a meta-analysis of experimental warming effects on 13 variables related to terrestrial N pools and dynamics.
- We found that, on average, net N mineralization and net nitrification rate were increased by 52.2 and 32.2%, respectively, under experimental warming treatment. N pools were also increased by warming, although the magnitude of this increase was less than that of N fluxes.
- Soil microbial N and N immobilization were not changed by warming, probably because microbes are limited by carbon sources. Grassland and shrubland/heathland were less responsive to warming than forest, probably because the reduction of soil moisture by warming offset the temperature effect in these areas. Soil heating cable and all-day treatment appeared to be the most effective method on N cycling among all treatment methods.
- Results of this meta-analysis are useful for better understanding the response of N cycling to global warming and the underlying mechanism of warming effects on plants and ecosystem functions.

Introduction

Global air temperature is predicted to increase by 2–5°C in the 2090s compared with preindustrial values Rogelj et al., 2012. Air temperature is generally positively correlated with soil temperature, although increased air temperature may lead to soil cooling during the winter (Groffman et al., 2001). Snowpack insulates the soil from the cold air and its decrease by warming could reduce soil temperature (Wisser et al., 2011). Besides soil temperature, warming may also have indirect effects on terrestrial ecosystems by reducing soil moisture (Brzostek et al., 2012), changing plant species composition (Saxe et al., 2001), and affecting the length of the growing season (Post et al., 2009). Although many studies suggested that direct warming effects on soil temperature were more important than indirect effects on soil moisture (Parton et al., 2007; Dijkstra et al., 2010; Cantarel et al., 2012), soil moisture becomes a limiting factor of N cycling in semiarid and arid regions, and the warming effect on microbial activities could be offset by the reduction in soil moisture (Liu et al., 2009; Verburg et al., 2009). Therefore, field manipulation experiments integrate a variety of these abiotic factors and could better reflect the effects of warming on terrestrial ecosystems.

Experimental warming has been carried out around the world in the past 20 yr, but much attention has been paid to the influence of warming on global carbon (C) cycle, especially on ecosystem net primary productivity (NPP) and soil respiration (Melillo et al., 2002; Eliasson et al., 2005; Hopkins et al., 2012). As one of the most limiting nutrients in terrestrial ecosystems, nitrogen (N) plays an important role in C cycle and climate change (Gruber & Galloway, 2008). As a result of the coupling of C and N during primary production, if a warming-induced increase in plant C storage is not accompanied by an increase in plant N, progressive N limitation may occur (Luo et al., 2004; Wu et al., 2012). A comprehensive evaluation of the effects of warming on ecosystem N pools and dynamics is urgently needed.

Elevated temperature may increase microbial metabolism and enzyme activity (Cookson et al., 2007; Feng et al., 2007; Koch et al., 2007), resulting in greater organic matter decomposition and gross N mineralization. While gross N mineralization is increased, microbial immobilization and plant uptake of N may also be increased. In laboratory incubation experiments, net N mineralization has been found to increase (Pendall et al., 2004) or remain unchanged (Niklińska et al., 1999) as temperature increases. A meta-analysis revealed that field warming increased net N mineralization rates by 46% (Rustad et al., 2001). However, Beier et al. (2008) found little influence of temperature on N mineralization across a range of shrubland ecosystems. We
hypothesize that these results differed because warming effects on N cycling are different in different ecosystems as a result of the interaction of temperature with other abiotic factors.

For nitrification and denitrification, the optimum temperature for the microbes in pure culture is believed to be 25–30°C (Saad & Conrad, 1993). So if warming brings soil temperature closer to the optimum temperature, activities of the nitrifier and denitrifier are expected to increase. However, Barnard et al. (2005) concluded from a meta-analysis that increased temperature had no effect on nitrifying enzyme activity (NEA) or net nitrification. One potential reason for this is that soil temperature could be elevated above the optimum temperature in tropical areas. An alternative explanation is that soil moisture and other abiotic factors interact with soil temperature and affect the responses. This also points to the possibility that different ecosystems respond differently to warming.

Warming may affect N leaching losses by increasing NO₃ availability and frequency of soil freeze–thaw cycles over winter (Turner & Henry, 2010). Leaching and gaseous N emissions from nitrification and denitrification are the two pathways for ecosystem N loss (Bai & Houlton, 2009). If ecosystem N loss is increased by warming while N input remains unchanged, the stimulation of N cycling by warming may eventually cause a decrease of ecosystem N. Additionally, N₂O is a potent greenhouse gas and ozone-depleting agent that could have a positive feedback on global warming (Ravishankara et al., 2009).

To simulate global warming, different experimental warming methods have been used. These include open-top chamber (OTC), infrared (IR) radiator, heating cable, glasshouse, and IR-reflective curtain. Although an IR radiator is thought to simulate climate change predictions most accurately (Aronson & McNulty, 2009), each method has its advantages and disadvantages. Owing to their different heated areas and different regulations on soil moisture and other abiotic factors, different methods are hypothesized to have different effects. In addition, winter season warming may affect the snowpack and have different results from growing-season warming. Night-time warming was found to have a greater impact on photosynthetic capacity than daytime warming during the following light period as a result of an increase in dark respiration and a reduction in leaf carbohydrate concentration (Turnbull et al., 2002). It is therefore important to study how the different settings of warming experiments (e.g., warming method, duration, seasonality) have different effects on N fluxes and pools.

While numerous individual studies have been conducted to investigate the effects of experimental warming on N cycling processes and pools, a data synthesis is still unavailable. Here we compiled data of 528 observations from 51 individual studies and conducted a meta-analysis to identify the general pattern of the effects of experimental warming on terrestrial N pools (leaf N, soil N, soil inorganic N, and microbial biomass N) and fluxes (gross N mineralization, net N mineralization, nitrification, denitrification, N leaching, N₂O emission); to investigate the differences among different settings of warming experiments (e.g., warming method, duration, seasonality); and to analyze warming-induced temperature and moisture effects on these N pools and fluxes.

### Materials and Methods

#### Data compilation

We searched journal articles published before July 2012 using the Web of Science resource. The search terms were ‘warming’ and ‘nitrogen’ or ‘temperature’ and ‘nitrogen’. A total of 528 observations taken from 51 papers (Supporting Information, Table S1) were selected and the compiled database included 13 variables related to N pools and dynamics (Tables S2, S3). The selection criteria were as follows: laboratory incubation studies were not included and only field warming manipulation studies were selected; at least one of the selected variables were measured; for multifactorial studies, only control and warming treatment data were used and the interacting effects were excluded; means and sample sizes had to be reported.

For each study, we noted the experiment location, warming method, ecosystem type, treatment seasonality, treatment duration, and the response variables. We also collected soil moisture values of treatment and control plots if reported. Annual soil moisture was calculated as the total of monthly values. Data were extracted by Engauge software if the figure was used in the original publication. The standard deviation was either reported or calculated from the standard error and sample size. The number of treatment plots refers to the number of replicate experimental facilities (i.e., warming plots, glasshouses, and so on), rather than the number of samples per plot. Some studies used the number of samples per plot as the sample size in their original papers, whereas we counted the replicate of experimental facilities instead.

Each study was grouped into one of these five ecosystems: forest, shrubland, grassland, tundra, and cropland. Plant species were grouped into one of five types: trees, shrubs, grasses and ferns, mosses and lichens, and crops. There were five categories of warming methods: OTC, IR radiator, heating cable, glasshouse, and IR-reflective curtain. When a range of raised soil temperatures was provided, the median was used. When the daytime temperature was not raised, the raised night-time temperature was used. Treatment duration was calculated in months and, if the exact duration was not reported, the approximate duration was estimated based on the reported season. Treatment season was categorized into all year, growing season, and winter treatment. Daily treatment time was categorized into all-day, daytime, and night-time treatments. When soil variables were reported for multiple soil depths, they were treated as separate entries. The denitrification rate was the potential denitrification based on incubation methods.

#### Statistical analyses

As a metric for the response of N pools and fluxes to experimental warming, we used the natural log of the response ratio ($R$), defined as the ‘effect size’ (Hedges et al., 1999). For a given variable, $R$ was calculated as the ratio of its value in the warming treatment group ($X$) to that in the control group ($X_c$) (Eqn 1).
The log transformation was done to improve its statistical behavior:

\[
\log_e R = \log_e \left( \frac{X_1}{X_2} \right) = \log_e(X_1) - \log_e(X_2)
\]  
\text{Eqn 1}

The effect size \( \log_e R \) was assumed to follow normal distribution and was fitted by a Gaussian function (Curtis & Wang, 1998; Luo et al., 2006). Negative values of \( R \) (e.g., the variable was < 0) were not used. The variance of \( \log_e R \) was approximated using the following formula:

\[
v = \frac{s_i^2}{n_i X_i^2} + \frac{s_c^2}{n_c X_c^2}
\]  
\text{Eqn 2}

where \( s_i \) and \( s_c \) are the standard deviations for all comparisons in the treatment and control groups, respectively; \( n_i \) and \( n_c \) are the sample sizes for the treatment and control groups, respectively. A few studies did not report standard deviation and we calculated the average coefficient of variation (CV) within each data set, and then approximated the missing standard deviation by multiplying the reported mean by the average CV.

A nonparametric weighting function was used to weight individual studies (Hedges et al., 1999). For each study, the weighting factor \( w \) was calculated as the inverse of the pooled variance (1/\( v \)). When multiple observations were extracted from the same study, we adjusted the weights by the total number of observations per site. The final weight (\( w' \)) used in the analyses was:

\[
w' = w / n
\]  
\text{Eqn 3}

where \( n \) is the number of observations from the same study.

Therefore we obtained a weighted response ratio \( R' \) and a weighted effect size \( \log_e R' \):

\[
\log_e R' = w' \times \log_e R
\]  
\text{Eqn 4}

The mean effect size (\( \log_e R' \)) for all observations was estimated as:

\[
\log_e R' = \frac{\sum_i \log_e R'_i}{\sum_i w'_i}
\]  
\text{Eqn 5}

where \( \log_e R'_i \) and \( w'_i \) are \( \log_e R' \) and \( w' \) of the \( i \)th observation, respectively.

To determine if warming had a significant effect on a variable, we employed a fixed-effects model using MetaWin (Rosenberg et al., 2000). Confidence intervals (CIs) on the weighted effect size were generated using bootstrapping (9999 iterations). If the 95% CI values of the effect size for a variable did not overlap with 0, the effect of warming on the variable was considered to differ significantly between two treatments. For a better explanation, the mean effect size was transformed back to the percentage change caused by the experimental warming treatment:

\[
(\log_e R' - 1) \times 100\%
\]  
\text{Eqn 6}

We conducted a test to compare responses among ecosystem types and warming methods, using a framework similar to ANOVA (Gurevitch & Hedges, 1993). We also applied a continuous randomized-effects model meta-analysis to test the relationships between \( \log_e R \) and the duration of warming treatment, raised soil temperature by warming, and change in soil moisture as a result of warming. Variables with > 20 observations were selected. Statistical results were reported as total heterogeneity in \( \log_e R \) among studies (\( Q_T \)), the difference among group cumulative effect sizes (\( Q_E \)), and the residual error (\( Q_\varepsilon \)) (Rosenberg et al., 2000).

**Results**

The mean effect sizes of experimental warming on leaf N and soil inorganic N were 0.032 (95% CI: 0.005–0.063) and 0.179 (95% CI: 0.048–0.274), respectively, and their 95% CI did not cover zero (Fig. 1a), showing a significantly positive effect of warming on these N pools. The effect size on microbial N was -0.001, with a 95% CI between -0.053 and 0.042, and the effect size on soil N was 0.023, with a 95% CI between -0.005 and 0.045 (Fig. 1a). The mean effect sizes of experimental warming on net N mineralization and net nitrification were 0.420 (95% CI:
The effect of the IR radiator was also significant for soil inorganic N mineralization (mean effect size = 0.128; 95% CI: −0.023–0.324). N leaching did not respond to warming treatment (mean effect size = 0.022; 95% CI: −0.596–0.390). The number of observations was small (<20) for the study of experimental warming effects on gross mineralization, gross nitrification, potential denitrification, immobilization, and N fixation. Based on the current limited number of studies, we found a positive effect of experimental warming on potential denitrification (mean effect size = 0.444; 95% CI: 0.321–0.580), a negative effect of experimental warming on N fixation (mean effect size = −0.141; 95% CI: −0.339 to −0.091), and nonsignificant effects of experimental warming on those other processes (Fig. 1b).

Soil inorganic N was changed most by the soil heating cable method (mean effect size = 0.324; 95% CI: 0.193–0.447), and this was also the case for leaf N (mean effect size = 0.029; 95% CI: 0.001–0.069), net N mineralization rate (mean effect size = 0.533; 95% CI: 0.412–0.658) and net nitrification rate (mean effect size = 1.338; 95% CI: 1.027–1.825) (Fig. 2). The effect of the IR radiator was also significant for soil inorganic N (mean effect size = 0.188; 95% CI: 0.025–0.270), net N mineralization (mean effect size = 0.311; 95% CI: 0.138–0.585), and net nitrification (mean effect size = 0.248; 95% CI: 0.026–0.516), but was not significant for leaf N (mean effect size = 0.053; 95% CI: −0.010–0.128). The OTC method did not have a significant effect on soil inorganic N (mean effect size = 0.008; 95% CI: −0.116 to 0.435) or leaf N (mean effect size = 0.012; 95% CI: −0.095 to 0.084), but it had a significant effect on net N mineralization (mean effect size = 0.427; 95% CI: 0.246–0.611) (Fig. 2). Glasshouse and IR curtain methods were seldom used and their effects on these N pools and fluxes were not significant.

Different ecosystems showed different responses to warming (Fig. 2). For the response of soil inorganic N to experimental warming, tundra (mean effect size = −0.063; 95% CI: −0.190–0.067) and shrubland (mean effect size = 0.063; 95% CI: −0.024–0.169) did not undergo a significant change, while cropland (mean effect size = 0.340; 95% CI: 0.247–0.424), forest (mean effect size = 0.249; 95% CI: 0.056–0.438), and grassland (mean effect size = 0.029; 95% CI: 0.001–0.076) all significantly increased (Fig. 2a). The effect of warming on leaf N was only significant for tree species (mean effect size = 0.102; 95% CI: 0.059–0.126) and grass and fern species (mean effect size = 0.057; 95% CI: 0.007–0.107), but was not significant for shrubs, mosses and lichens, or crops (Fig. 2b). The increase of net N mineralization under warming was most significant in forest (mean effect size = 0.561; 95% CI: 0.444–0.692), followed by shrubland (mean effect size = 0.400; 95% CI: 0.147–0.624) and grassland (mean effect size = 0.127; 95% CI: 0.002–0.246). But net N mineralization was not changed by warming in tundra (mean effect size = 0.633; 95% CI: −0.811–1.164) based on the three observations. The warming effect on nitrification has been studied in grassland, shrubland, and forest ecosystems, and it was not significant in grassland (mean effect size = −0.001; 95% CI: −0.166–0.125), but it was significant in shrubland (mean effect size = 0.323; 95% CI: 0.277–0.412) and forest ecosystems (mean effect size = 1.097; 95% CI: 0.849–1.378).

Most studies adopted all-day warming treatments, but a few studies used night-time or daytime warming only (Table S2). Night-time warming treatment did not have a significant effect on leaf N (mean effect size = −0.049; 95% CI: −0.145–0.184), but it did have a significantly positive effect on soil inorganic N (mean effect size = 0.297; 95% CI: 0.047–0.567), N mineralization (mean effect size = 0.492; 95% CI: 0.393–0.634), and nitrification (mean effect size = 0.441; 95% CI: 0.273–0.542) (Fig. 2). There were only two observations for daytime warming treatments, which were not enough for evaluation.

Seasonally, experimental warming has been carried out for all-year, growing season, or winter season periods (Tables S2, S3). While all-year warming had a significant effect on soil inorganic N (mean effect size = 0.193; 95% CI: 0.042–0.281), leaf N (mean effect size = 0.045; 95% CI: 0.004–0.086), net N mineralization (mean effect size = 0.442; 95% CI: 0.333–0.563), and net nitrification (mean effect size = 0.656; 95% CI: 0.415–0.923), growing-season warming only had a significant effect on net N mineralization (mean effect size = 0.544; 95% CI: 0.332–0.785). Winter warming resulted in a slightly higher increase in leaf N, soil inorganic N, and nitrification than did all-year warming, but the variations in the effect sizes were also higher as a result of the small number of observations (Fig. 2).

The continuous randomized-effects model meta-analyses showed a significantly negative correlation between warming duration and the effect size of warming on leaf N (P = 0.032, Table 1). Raised soil temperature had a significantly positive correlation with the effect size of warming on soil inorganic N, net N mineralization, and net nitrification (Table 1). The effect size of warming on soil moisture did not show any correlation with the effect sizes on N pools and fluxes (Table 1).

Soil moisture was generally reduced by experimental warming treatments (Fig. 3). Soil moisture was reduced in forest (mean effect size = −0.139; 95% CI: −0.185 to −0.107), grassland (mean effect size = −0.168; 95% CI: −0.261 to −0.068), and cropland (mean effect size = −0.040; 95% CI: −0.077 to −0.012) ecosystems, but was unchanged in shrubland/heathland ecosystems (mean effect size = 0.001; 95% CI: −0.074–0.046). The IR warming method caused the greatest reduction in soil moisture (mean effect size = −0.157; 95% CI: −0.242 to −0.067) while the glasshouse method had the least effect (mean effect size = 0.048; 95% CI: −0.036–0.133). Winter-season warming had the biggest effect on soil moisture (mean effect size = −0.084; 95% CI: −0.122 to −0.029) compared with all-year and growing-season warming (Fig. 3).

**Discussion**

**Overview**

Our meta-analysis indicated that experimental warming increased soil inorganic N by 20.0% and increased leaf N by 3.3%, but did not change soil total N or microbial N (Fig. 4). For N fluxes,
warming increased net N mineralization by 52.2%, net nitrification by 32.2%, and potential denitrification by 55.9%, but did not significantly change gross mineralization, N leaching, or N immobilization (Fig. 4). N2O emission was increased by 14.0%, although the effect was not statistically significant. Raised soil temperature by experimental warming was significantly correlated with the increase in net N mineralization and net nitrification, showing a direct temperature effect on these fluxes. While the soil inorganic N pool responded significantly to raised soil temperature, leaf N and soil N did not show any correlation with raised soil temperature (Table 1). This is probably because these pools are much larger than the soil inorganic N pool (Galloway et al., 2008) and it may take much longer for them to respond to warming. The change in soil moisture by warming did not show any correlation with changes in N pools or fluxes because the moisture effect was overshadowed by the temperature effect under warming (Table 1).

Nitrogen fixation was decreased by warming based on the existing four observations. The decreased N fixation, together with increased N uptake and denitrification, should cause a

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**Fig. 2** The mean effect sizes of experimental warming on soil inorganic nitrogen (N) (a), leaf N (b), net mineralization (c), and net nitrification (d). The variables are categorized into different groups depending on the warming methods, ecosystem types, warming time, and warming season. Error bars represent 95% confidence intervals (CIs). The dashed line was drawn at mean effect size = 0. The effect of warming was considered significant if the 95% CI of the effect size did not cover zero. The sample size for each variable is shown next to the point. OTC, open-top chamber.
decrease of soil N. However, our meta-analysis showed an increase of soil N as a result of warming, although the effect was not significant. The potential reasons for the discrepancy are the limitation of meta-analyses (each pool or flux was analyzed independently in meta-analyses and the responses may be unconnected) or the limited number of experiments (there was only one study with four observations investigating warming effects on N fixation and the result may be biased). Overall, forest ecosystems showed the strongest response to warming of all the ecosystems (Fig. 2). Rustad et al. (2001) also found that forest ecosystems had the strongest response to warming in terms of soil respiration. The results indicated either that N cycling in forest ecosystems is more cold-limited or that the studies in forest ecosystems have mostly been done in cold-limited areas. We also found that the relationship between the effect sizes on soil moisture and nitrification for grassland/prairie ecosystems was significant ($P < 0.001$). The reduction in soil moisture as a result of warming in grassland/prairie ecosystems (Fig. 3) may offset the temperature effect because these systems became drier and more moisture-limited under warming. Therefore, of the five ecosystems, it is N cycling in forests that is projected to be most affected by future global warming.

The heating cable method appeared to be most effective warming method, followed by IR radiation (Fig. 2). Heating cables provided the most homogenous warming throughout the soil strata (Rustad & Fernandez, 1998) and directly increased soil temperature. IR lamps simulated climate change predictions most accurately (Aronson & McNulty, 2009) but
reduced soil moisture to the greatest extent (Fig. 3). Glasshouse and OTC methods are passive warming methods and they only increased net N mineralization rate while not affecting soil inorganic N, leaf N, or nitrification (Fig. 2). For these passive warming methods, the variability in the temperature difference between control and treatment chambers can be very large; and photosynthesis and herbivory level may be more confounded due to the establishment of the chamber walls (Aronson & McNulty, 2009). However, passive methods had less influence on soil moisture (Fig. 3). Night-time and all-day warming both increased soil inorganic N, net N mineralization and nitrification, and the magnitudes were similar (Fig. 2). All-year warming appeared to be more effective in affecting soil inorganic N, leaf N, and net nitrification than growing-season warming (Fig. 2).

Gross and net N mineralization

On average, warming increased gross N mineralization, although nonsignificantly (Fig. 1), and it significantly increased net N mineralization rates by 52.2% (Fig. 4). Because there were only three studies on gross N mineralization, we focused on net N mineralization, for which there were 69 observations from 19 studies. We found a positive correlation between raised soil temperature and the effect of warming on net N mineralization rate (Table 1), indicating a direct temperature effect. Some studies found the increase in net N mineralization to be sustained for a long time after the warming treatment (Melillo et al., 2002; Butler et al., 2012), whereas others found interannual variability in net N mineralization (Wan et al., 2005). Stimulated plant growth and enhanced microbial immobilization could be the cause of decreased net N mineralization after a certain period of warming treatment, though other studies (Shaw & Harte, 2001; Carrillo et al., 2012) believed soil moisture and/or vegetation type were the reasons for these temporal variations. Our meta-analysis did not find that warming treatment duration or change in soil moisture had an impact on the change in net N mineralization as a result of warming (Table 1).

The warming effect on net N mineralization was not significant in grassland and tundra ecosystems in our study (Fig. 2c), which may be attributed to one of the following reasons: the number of studies in these ecosystems was limited; the greater reduction in soil moisture as a result of warming (Fig. 3) in these ecosystems offset the temperature effect (Brzostek et al., 2012); the decrease in plant below-ground C under warming (Lu et al., in press) limited microbial activities in these ecosystem; or acclimation of physiological processes occurred in these ecosystems (Atkin & Tjoelker, 2003; Davidson & Janssens, 2006), although the underlying mechanisms are still not clear. We believe the interaction between temperature and other abiotic factors, especially moisture, is an important determinant. For example, Brzostek et al. (2012) suggested that warming stimulated potential activity of proteolytic enzyme in mesic sites – temperate and boreal forests and arctic tundra – but suppressed its potential activity in dry grasslands. In summary, our meta-analysis indicated a significant stimulation effect of experimental warming on net N mineralization, the magnitude of which is ecosystem-dependent.

Microbial N and N immobilization

We did not find a significant effect of warming on microbial N immobilization or microbial N (Fig. 1). Warming may reduce labile C for microbes and have a negative effect on microbial biomass (Bradford et al., 2008; Frey et al., 2008; Butler et al., 2012), whereas greater N availability could increase microbial N (Yin et al., 2012). Our meta-analysis reflected the balanced result of these two factors. Water stress (Fierer et al., 2003), heat stress (Banning & Murphy, 2008; Wittebolle et al., 2009), or change of microbial community composition (Dalias et al., 2001; Zhang et al., 2005) under warming may also potentially influence microbial biomass and N immobilization.

Gross and net nitrification

Warming significantly increased net nitrification rates, although its effect on gross nitrification rates was not significant (Fig. 1). Because there were only two studies on gross nitrification, we focused on net nitrification. The optimum temperature for nitrification is expected to increase if warming brings soil temperature closer to the optimum, but may also be inhibited if the temperature rises higher than the optimum. We found a positive correlation between raised soil temperature and the effect of warming on net nitrification rate (Table 1), showing a direct temperature effect (Table 1). Increased temperature may also induce microbial community shift (Avrahami & Conrad, 2003) that could influence nitrification rate. The increase of net N mineralization by warming (Fig. 1) also supports the enhancement of nitrification because these factors have been found to be positively correlated (Owen et al., 2003; Venterea et al., 2003).

Our meta-analysis indicated that the change of nitrification rates by warming was most significant in forest ecosystems, whereas it was nonsignificant in grassland/prairie ecosystems (Table 1). We speculate that the relatively dry condition in grasslands may play a role in this difference. The nitrification rate is highest at moderate moisture contents (water-filled pore space (WFPS) is c. 55%) (Szukics et al., 2010). If decreased soil moisture as a result of warming (Fig. 3) drives WFPS closer to this value, the nitrification rate may increase; otherwise, it may decrease.

Denitrification

Warming significantly increased potential denitrification rates based on our meta-analysis (55.9%, Fig. 4). However, there were
only seven observations available. Denitrification is an anaerobic process that is influenced by temperature, oxygen, readily available carbon, and nitrate availability (Tiedje, 1988). Laboratory incubation studies found that increased temperature resulted in the enrichment of denitrifiers and shifts in community composition, causing an increase in denitrification (Braker et al., 2010). Warming could also indirectly affect denitrification by changing the availability of N and C substrates and soil oxygen concentration (Loiseau & Soussana, 2000; Barnard et al., 2005). Enhanced nitrification rates as a result of warming yielded a greater amount of substrate for potential denitrification (Fig. 1). Lower soil moisture caused by warming could promote nitrification and reduce denitrification as a result of changes in soil aeration and O2 content (Smith et al., 2003). On the other hand, increased soil respiration under warming consumes more O2, favoring denitrification (Castaldi, 2000). Increased dissolved organic carbon (DOC) content under warming also provides more C for denitrification (Tscherko et al., 2001).

**N2O emission and N leaching**

The 26 observations of N2O emissions under warming suggested a 14% increase, although this was not statistically significant (Fig. 1). Soil N2O is mainly from nitrification and denitrification, and is highly spatially and temporally variable as a result of the intricacy of nitrification and denitrification processes and the complex regulation of their controlling factors (Groffman et al., 2009). Our results indicated that higher temperatures increased N availability (Fig. 1), which could result in a positive response of N2O to warming (Bijoor et al., 2008). The contribution of nitrification and denitrification to N2O emissions has been shown to be sensitive to temperature in microcosm studies (Maag & Vinther, 1996; Gödde & Conrad, 1999; Stres et al., 2008). Maag & Vinther (1996) showed that higher temperature decreased nitrification-derived N2O fluxes, but increased denitrification-associated N2O fluxes. In addition, reduced moisture by warming generally has a negative effect on denitrification-derived N2O and a positive effect on nitrification-derived N2O (Bijoor et al., 2008). Dijkstra et al. (2012) also suggested in their review that warming affected N2O emission fluxes variably. Therefore, how N2O fluxes respond to warming is complex and should be studied carefully under different conditions.

The warming effect on N leaching was nonsignificant (Fig. 3). N leaching is highly affected by precipitation, soil water-holding capacity and drainage conditions (Thorup-Kristensen et al., 2009). Warming may decrease soil moisture in dry areas, but to a much smaller extent than the impact of precipitation (Patil et al., 2010). However, it is worth noting that most leaching observations were done in rather wet environments. More studies should be carried out in other ecosystems to see how warming would affect N leaching.

**Soil N**

On average, both soil inorganic N and soil total N were increased by warming, although their magnitudes were different (Fig. 4). Net N mineralization and nitrification were found to increase with warming (Fig. 1), which should be reflected as an increase in soil inorganic N. It should be noted that soil inorganic N was reduced by warming in tundra ecosystems, although the effect was not significant (Fig. 2a). This may be related to increased plant N uptake or increased N leaching affected by the snowpack in tundra ecosystems.

**Leaf N**

Warming increased leaf N by 2.8% (Fig. 4). The increase of soil N availability (Fig. 1) and plant N uptake may have contributed to this phenomenon. Studies have found that N uptake increased with temperature until the optimum was reached, if no other factors were limiting (Gessler et al., 1998; Bassirirad, 2000; Dong et al., 2001; Weih & Karlsson, 2002). For example, Gessler et al. (1998) found that 25°C was the optimum temperature of nitrate uptake for pre-alpine spruce and beech trees, which was higher than the soil temperature most of the time in the area. Alternative explanations include an increase in above-ground allocation at the expense of below-ground allocation as a result of warming (Fan et al., 2009), or a decrease in leaf biomass with no change in N uptake.

Leaf N is positively correlated with plant photosynthetic capacity across all biomes (Reich et al., 1994, 1995; Ollinger et al., 2008; Kattge et al., 2009). For a given species, leaf N has also been found to be positively correlated with photosynthetic capacity (Ellsworth & Reich, 1992). Enhanced carbon assimilation resulting from an increase in leaf N may attenuate global warming caused by elevated CO2. In the long run, this may lead to increased NPP and N demand, and ultimately to decreased N availability in the soil (Luo et al., 2004). The negative correlation between warming duration and the effect size of warming on leaf N (P = 0.032, Table 1) pointed to potential acclimation of leaf N to warming.

**Conclusions**

Warming effects on N pools and fluxes are interrelated and interdependent. Our meta-analysis has indicated that warming generally increased N cycling processes and soil and leaf N content, but did not affect microbial N. Increased net N mineralization, nitrification and soil inorganic N as a result of warming may lead to higher N losses from soil. N2O emission was increased slightly by warming associated with the increase in potential denitrification. Enhanced carbon assimilation as a result of the increase in leaf N may lead to increased NPP and N demand, and, if N losses increase over time under warming, progressive N limitation may occur. The influences of warming on these N fluxes and pools varied across different ecosystems. Global warming may affect forest ecosystems to the greatest extent. In drier grassland/prairie, the reduction of soil moisture as a result of warming could offset the direct temperature effect. The soil heating cable method appears to be the most effective of all the warming methods in relation to N cycling. In order to understand if acclimation is occurring, long-term field experiments studying the effects of
warming on microbial communities and N cycling are needed. A paucity of data exists regarding the effect of warming on soil N processes such as denitrification, immobilization, and N fixation. The results of this meta-analysis have provided a thorough understanding of the responses of N cycling processes to experimental warming, and should improve understanding of the underlying mechanisms of warming effects on plants and ecosystem functions.

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References


Supporting Information
Additional supporting information may be found in the online version of this article.

Table S1 List of all the references used in the meta-analysis

Table S2 Summary of the references and data used in the meta-analysis of warming effects on nitrogen fluxes and pools

Table S3 Data used in the meta-analysis and calculation of the effect size of warming on each variable

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