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Responses of terrestrial nitrogen pools and dynamics to different patterns of freeze-thaw cycle: A meta-analysis

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Abstract

Altered freeze-thaw cycle (FTC) patterns due to global climate change may affect nitrogen (N) cycling in terrestrial ecosystems. However, the general responses of soil N pools and fluxes to different FTC patterns are still poorly understood. Here, we compiled data of 1519 observations from 63 studies and conducted a meta-analysis of the responses of 17 variables involved in terrestrial N pools and fluxes to FTC. Results showed that under FTC treatment, soil NH_4^+, NO_3^-, NO_3^- leaching, and N_2O emission significantly increased by 18.5%, 18.3%, 66.9%, and 144.9%, respectively; and soil total N (TN) and microbial biomass N (MBN) significantly decreased by 26.2% and 4.7%, respectively; while net N mineralization or nitrification rates did not change. Temperate and cropland ecosystems with relatively high soil nutrient contents were more responsive to FTC than alpine and arctic tundra ecosystems with rapid microbial acclimation. Therefore, altered FTC patterns (such as increased duration of FTC, temperature of freeze, amplitude of freeze, and frequency of FTC) due to global climate warming would enhance the release of inorganic N and the losses of N via leaching and N_2O emissions. Results of this meta-analysis help better understand the responses of N cycling to FTC and the relationships between FTC patterns and N pools and N fluxes.

KEYWORDS
denitrification, freeze-thaw cycle, microbial biomass, mineralization, N fluxes, N_2O, nitrification

1 | INTRODUCTION

The freeze-thaw cycle (FTC) is a manifestation of the dynamics of soil heat, which is the repeated freezing-thawing process caused by the phase transition of soil water. This phenomenon is very common in the high altitude, high latitude, and some temperate regions and about 55% of the total land areas in the northern hemisphere are subject to seasonal soil freezing (Grogan, Michelsen, Ambus, & Jonasson, 2004; Kreyling, Beierkuhnlein, Pritsch, Schloter, & Jentsch, 2008; Zhang, Barry, Knowles, Ling, & Armstrong, 2003). Global climate warming is predicted to cause milder winter, thinner and more unstable snow cover in terrestrial ecosystems (Wang, Shu, Zhang, & Guenon, 2015), which may alter

FTC regimes. Milder winter may decrease soil freezing in regions without snow cover; but because snow serves as an insulation layer (Brooks et al., 2011; Wisser, Marchenko, Talbot, Treat, & Frolking, 2011), in areas with snow, milder winter may decrease the snow amount and hence increase the intensity, frequency, and duration of FTC. These changes in FTC regimes may strongly affect soil structure (Oztas & Fayetorbay, 2003; Six, Bossuyt, Degryze, & Denef, 2004), soil microorganisms (Larsen, Jonasson, & Michelsen, 2002; Yanai, Toyota, & Okazaki, 2004), plant fine roots (Campbell, Socci, & Templer, 2014; Gaul, Hertel, & Leuschner, 2008; Reinmann & Templer, 2016), and plant litter inputs (Pelster et al., 2013; Su, Kleineidam, & Schloter, 2010), which all may influence soil nutrient cycling.

Soil nitrogen (N) as an important nutrient plays an important role in plant growth, especially in winter and the early growing season (Tan, Wu, Yang, & He, 2014; Ueda, Muller, Nakamura, Nakaji, & Hiura, 2013). Recently, Song, Zou, Wang, and Yu (2017) explored the effects of FTC on soil carbon (C) and N cycles using the meta-analysis method, focusing on the major soil C and N variables under different environmental factors. However, to date, the specific effects of the different FTC patterns on soil N pools and fluxes are rarely comprehensively analyzed.

Soil NH_4^+ was found to increase under FTC mainly due to the release from broken soil aggregates (Oztas & Fayetorbay, 2003; Six et al., 2004), the killed soil microbes (Larsen et al., 2002; Yanai et al., 2004), and the reduced uptake by damaged plant roots (Campbell et al., 2014; Reinmann & Templer, 2016). With the increase in soil NH_4^+, soil NO_3^- may also increase (Campbell et al., 2014). However, with increasing intensity, frequency, and duration of FTC, whether the effects would be more severe is still unclear.

Previous studies found contradicting responses of N-cycling processes to FTC events, including increased net N mineralization (Herrmann & Witter, 2002), and denitrification (Neilsen et al., 2001), decreased net N mineralization (Zhao, Zeng, & Fan, 2008), nitrification (Zhao et al., 2008) and denitrification (Neilsen et al., 2001), or no significant change in net N mineralization (Hentschel et al., 2009) and nitrification (Neilsen et al., 2001).

Soil microbes as the important drivers of the N-cycling processes also showed different responses to FTC events. For example, soil microbes were found to have strong acclimation in colder regions, resulting in unchanged microbial biomass N (MBN) under FTC in tundra (Grogan et al., 2004; Larsen et al., 2002; Lipson, Schmidt, & Monson, 2000), while in contrast, MBN reduced under FTC in temperate regions (Groffman et al., 2011). These discrepancies might be due to the different intensity, frequency, and duration of different FTC treatments and should be further explored.

Soil N losses including gaseous N emissions and N leaching may increase under FTC due to the enhanced inorganic N availability and other potential effects of FTC (Campbell et al., 2014). The increase in soil NO_3^- as a result of FTC may increase the risk of N leaching losses due to the mobility of NO_3^-. In addition to the potential increase in denitrification processes, the physical release of stored gases in snow and soil pores could also cause an increase in gaseous N emissions. A burst of soil N_2O emission during the FTC periods has been observed in many studies (Cui et al., 2016; Neilsen et al., 2001; Teepe & Ludwig, 2004) and severe freeze induced larger effects than mild freeze (Sulkava & Huhta, 2003). However, N_2O emission in arable soils was found to reduce with a series of FTC (Koponen & Martikainen, 2004). These N losses would cause important consequences on ecosystem productivity (Henry, 2007), aquatic eutrophication, ozone damage, greenhouse effects, and photochemical smog, etc. (IPCC, 2013; Ravishankara, Daniel, & Portmann, 2009). Therefore, the assessment of N loss fluxes during the FTC periods is important for many aspects.

While numerous individual studies have been conducted to investigate the responses of ecosystem N pools and dynamics to FTC, a metadata synthesis would reveal the general pattern globally and explore the potential effects of changing FTC pattern under the contexts of global changes. In this study, 1519 individual experimental observations were compiled from 63 peer-reviewed publications. The objectives of our study were as follows: (i) to identify the general patterns of the responses of terrestrial N pools and fluxes to FTC events across different ecosystems; and (ii) to investigate the different effects under different settings of FTC experiments to better predict the responses of N pools and dynamics to changing intensity and frequency of FTC.

2 | MATERIALS AND METHODS

2.1 | Data compilation

We collected data from peer-reviewed journal articles published before October 2016 using the Web of Science resource. The search terms were "freeze thaw" and "nitrogen" or "freeze thaw" and "nitrous oxide". A total 1519 observations of 17 variables (Tables S1, S2) related to N pools and dynamics were taken from 63 papers (Table S3). The following criteria were applied to select proper studies: (i) at least one of the selected variables was measured; (ii) for multifactorial studies, only the control and FTC treatment were selected and the interacting effects were excluded; (iii) the means and sample sizes had to be reported.

For each study, we noted the 17 N variables (TN (soil total N), DTN (soil dissolved total N), DIN (soil dissolved inorganic N), DON (soil dissolved organic N), NH_4^+, NO_3^-, MBN, NH_4^+ leaching, NO_3^- leaching, DON leaching, TDN leaching, N_2O, NO, Net N mineralization, nitrification, ammonification, and denitrification), nine experimental settings (FTC method, ecosystem type, FTC duration, freeze duration, thaw duration, freeze temperature, thaw temperature, FTC amplitude, and FTC frequency), and some other background information (e.g., study location, soil moisture, pH, soil type, mean annual precipitation, and mean annual temperature). To visualize the global distribution of FTC experiments selected in this meta-analysis, the study sites were marked on the map. The study sites mainly distributed in China, Japan, North America, and Western Europe (Figure 1). Data were extracted using Getdata software (http://www.getdata-graph-digitizer.com/index.php) if a figure was used in the original publication. The standard deviation was either reported or calculated from the standard error and sample size ($SD = SE\sqrt{n}$).

The FTC method was grouped into either laboratory incubations or field experiments (including in situ incubation, heating wires, and snow removal). Each study was grouped into one of these ecosystems: temperate forest, alpine forest, boreal forest, temperate grassland, alpine grassland, shrubland, cropland, wetland, and tundra. The FTC frequency was categorized into low (≤5 times), medium (6~15 times), and high frequency (>15 times).

2.2 | Statistical analyses

The natural log of the response ratio (R), defined as the "effect size," was used as a metric to weigh the response of N pools and fluxes to
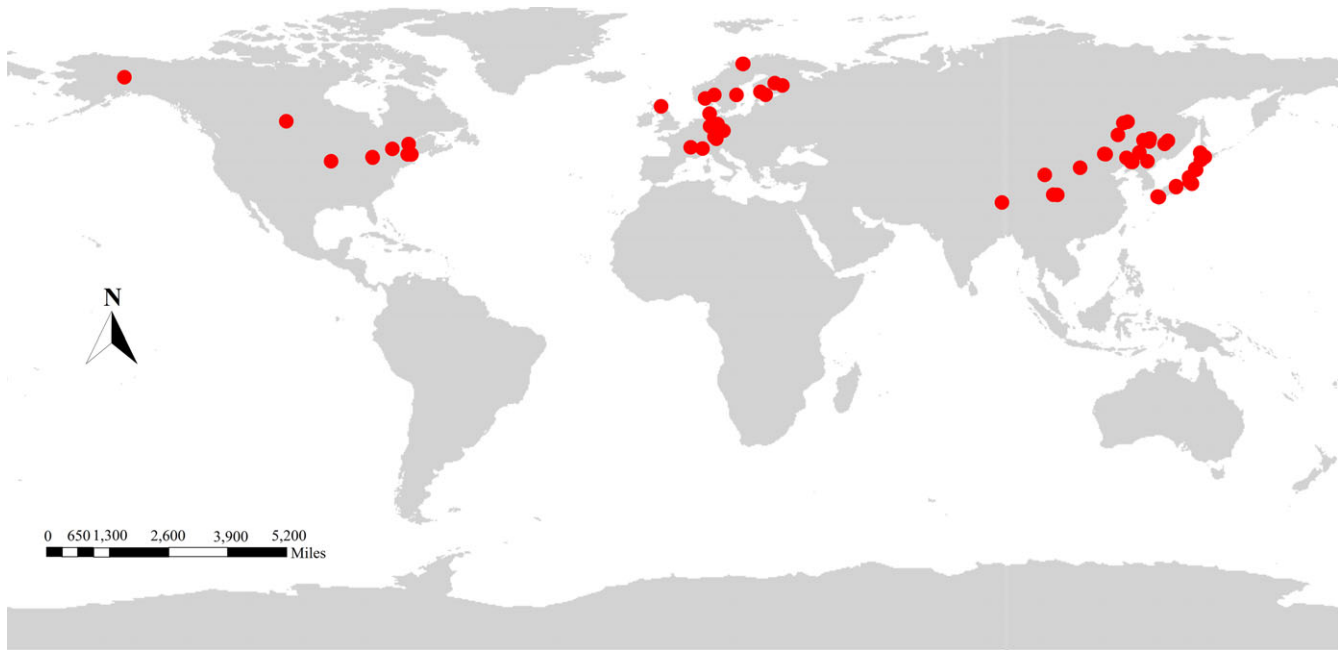
FIGURE 1 The distribution of the freeze-thaw cycle selected in this meta-analysis in the world

FTC (Hedges, Gurevitch, & Curtis, 1999). R was calculated as the ratio of its value under the FTC treatment ($\bar{X}_t$) to that under the control treatment ($\bar{X}_c$) (Equation 1). The log transformation was carried out to improve its statistical behavior in meta-analyses:

$$\ln R = \ln\left(\frac{\bar{X}_t}{\bar{X}_c}\right) = \ln(\bar{X}_t) - \ln(\bar{X}_c) \tag{1}$$

The variable was not used if it was less than zero. The variance of ln R (v) was approximated using the following formula:

$$v = \frac{s_t^2}{n_t \bar{x}_t^2} + \frac{s_c^2}{n_c \bar{x}_c^2} \tag{2}$$

where S_t and S_c are the standard deviation for the FTC treatment and the control, respectively; n_t and n_c are the sample sizes for the FTC treatment and the control, respectively. If neither standard deviation nor standard error was reported, the missing standard deviation was estimated by multiplying the reported mean by the average coefficient of variation (CV) with each data set (Wiebe et al., 2006).

The weighting factor (w) of each observation was calculated as the inverse of the pooled variance (1/v). To reduce the weight of many studies from the same site, the weight was adjusted by the total number of observations per site if a study contained two or more observations for one variable. The final weight (w') was calculated according to Equation 3 (Bai et al., 2013; Liu et al., 2016).

$$w' = w/n \tag{3}$$

where n is the total number of observations from the same study.

Finally, the mean ln R' ($\overline{\ln R'}$) of all observations was estimated as follows:

$$\ln R' = w' \times \ln R \tag{4}$$

$$\overline{\ln R'} = \frac{\Sigma_i \ln R_i'}{\Sigma_i w_i'} \tag{5}$$

where ln R' is the weighted ln R, ln R_i' and w_i' are ln R' and w' of the ith observation, respectively.

We used a linear full factorial model considering the effects of "method," "ecosystem type," and "frequency of FTC" and their interactions on ln R'. As the purpose of a meta-analysis is to find the general pattern worldwide, we only presented and discussed the main effects (methods, ecosystem type, and frequency of FTC) although the interaction effects were considered. Estimated marginal means and the bootstrap 95% confidence interval (CIs) for each factor were presented. Bootstrapping with 9999 iterations was used to generate the CIs of the weighted ln R. If the 95% CI values of the ln R' for a variable did not overlap with 0, FTC treatment had a significantly positive or negative effect on the variable. To facilitate explanation, $\overline{\ln R'}$ was transformed back to the percentage change resulted from the FTC treatment using the following formula:

$$(e^{\overline{\ln R'}} - 1) \times 100\% \tag{6}$$

A continuous randomized-effect model meta-analysis (using Metawin software (2.1) (Rosenberg, Adams, & Gurevitch, 2000)) was used to test the relationships between the ln R' of N variables and the following experimental setting variables: the duration of FTC, the duration of freeze, the duration of thaw, the temperature of freeze, the temperature of thaw, the amplitude of FTC, the frequency of FTC, and soil moisture. The N-related variables were selected only when the number of observations was more than 20. We reported the statistical results as total heterogeneity of ln R' among studies

(Q_T), the difference among group cumulative ln R (Q_M) and the residual error (Q_E) (Rosenberg et al., 2000).

3 | RESULTS

3.1 | Mean effect size of FTC on N pools and fluxes

For soil N pools, $\overline{\ln R'}$ of FTC on DIN, NH_4^+, and NO_3^- were 0.177, 0.169, and 0.168, respectively, and their 95% CIs did not overlap with zero (Figure 2), showing a significantly positive effect of FTC on these variables. FTC significantly decreased TN and MBN, but did not affect DTN or DON.

For soil N losses, among the variables with more than 20 observations, soil N_2O emission was most significantly enhanced by FTC, and $\overline{\ln R'}$ was 0.896 with a 95% CI between 0.653 and 1.506. $\overline{\ln R'}$ of FTC on NO_3^- leaching and DON leaching were 0.512, and 0.312, respectively, and their 95% CI did not cover zero (Figure 2), revealing a significantly positive effect of FTC on these variables. NH_4^+ leaching was also increased by FTC, but the effect was not significant. Soil NO emission was significantly reduced by FTC. Based on the current limited number of studies on TDN leaching (17 observations), we found a positive response of TDN leaching to FTC (Figure 2).

For soil N processes, FTC increased soil net N mineralization and nitrification, although these effects were not statistically significant (Figure 2). According to the current limited number of studies on soil ammonification (six observations) and denitrification (19 observations), we found a significantly positive effect of FTC on ammonification and a nonsignificant effect of FTC on denitrification (Figure 2).

### 3.2 | Responses of N pools and fluxes to FTC under different experimental methods

Different experimental methods showed different responses to FTC (Figures 3–4). For laboratory incubation method, soil NH_4^+, NO_3^-, DIN, DON, N_2O emission, NH_4^+ leaching, and NO_3^- leaching significantly enhanced under FTC. MBN significantly diminished under FTC, but FTC did not affect DTN, net N mineralization, or net nitrification (Figures 3–4).

For field transplant incubation (in situ) method, soil NO_3^- leaching dramatically decreased under FTC based on the two observations. Soil NH_4^+, DIN, MBN, net N mineralization, net nitrification, N_2O, and NH_4^+ leaching markedly increased under FTC, but FTC did not affect soil NO_3^-, DON, or DTN (Figures 3–4).

For field snow removal method, FTC pronouncedly enhanced soil NH_4^+, N_2O emission, and NO_3^- leaching, but did not have impact on soil NO_3^-, DON, MBN, net N mineralization, net nitrification, or NH_4^+ leaching (Figures 3–4). Field burying heated wires method was seldom used and the two observations showed nonsignificant effect of FTC on soil NO_3^- (Figure 3b).

3.3 | Responses of N pools and fluxes to FTC in different ecosystems

Soil N pools and fluxes showed various responses to FTC in different ecosystems (Figures 3–4). The increase in soil NH_4^+ under FTC was significant in alpine forests, temperate grasslands, alpine grasslands, croplands, and wetlands, but was not significant in temperate forests and tundras (Figure 3a). The effect of FTC on soil NO_3^- was
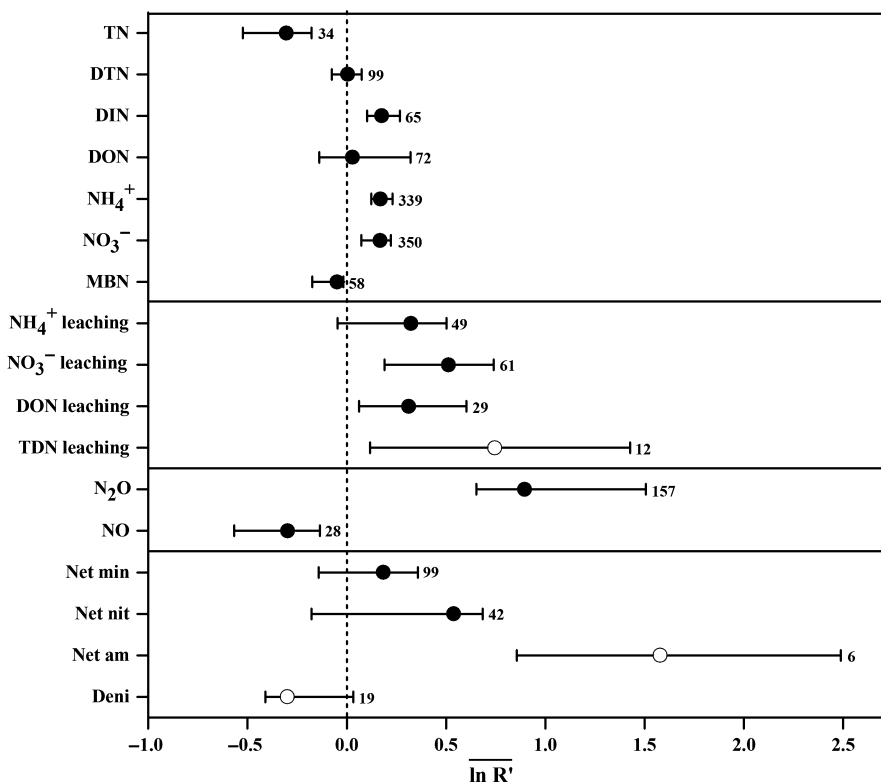
FIGURE 2 The mean effect size $(\overline{\ln R'})$ of freeze-thaw cycle (FTC) on seven variables related to nitrogen (N) pools and ten variables related to N fluxes. Error bars stand for 95% bootstrapped confidence intervals (CIs). Solid points are variables with >20 observations, and hollow points are variables with <20 observations. If the 95% CI of the $\overline{\ln R'}$ did not overlap with zero, a significant FTC effect was considered. The sample size for each variable is shown next to the error bars. TN, soil total nitrogen; DTN, soil dissolved total nitrogen; DIN, soil dissolved inorganic nitrogen; DON, soil dissolved organic nitrogen; MBN, soil microbial biomass nitrogen; min, mineralization; nit, nitrification; am, ammonification; Deni, denitrification
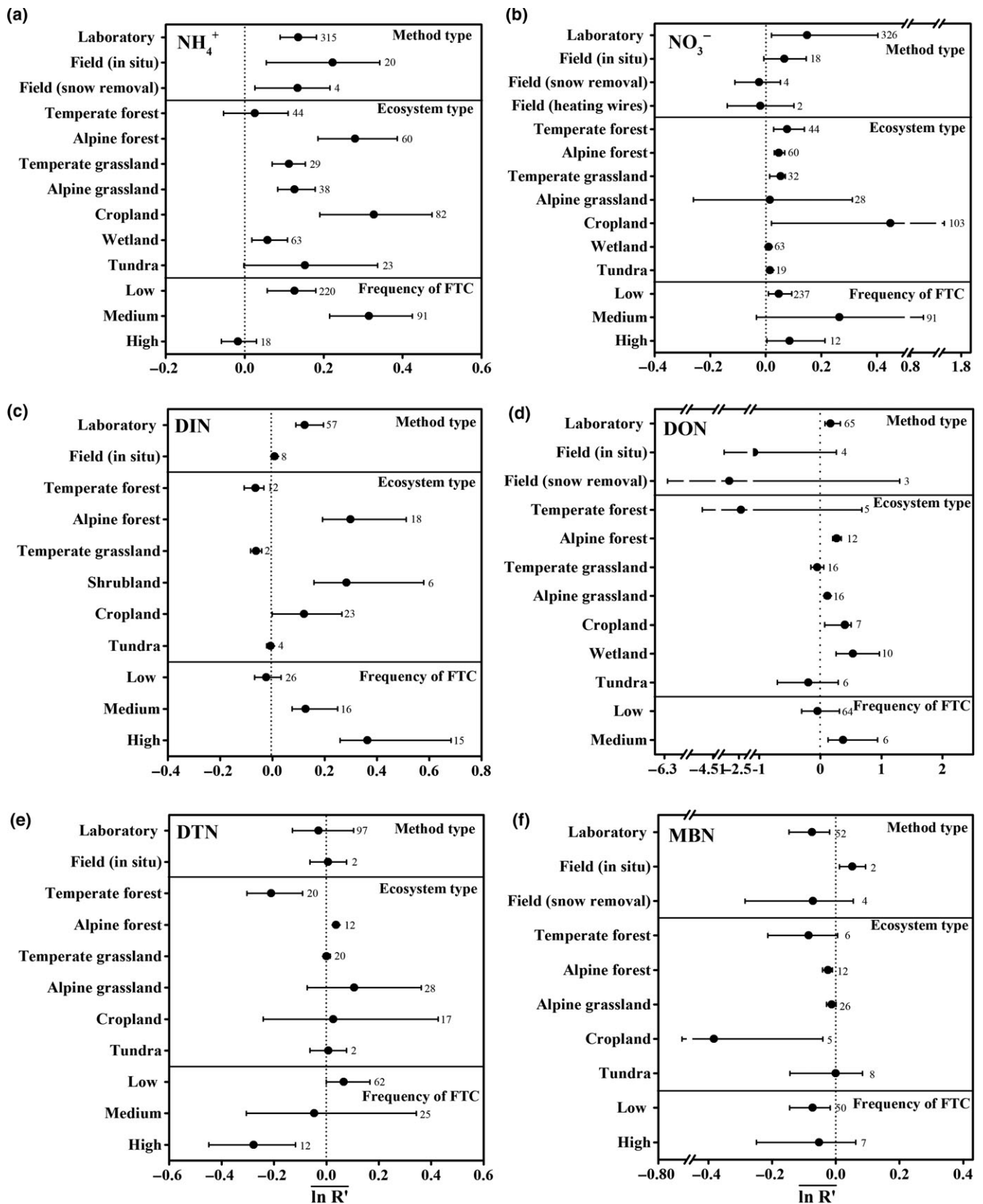
FIGURE 3 The mean effect size ($\overline{\ln R'}$) of freeze-thaw cycle (FTC) on soil NH_4^+ (a), soil NO_3^- (b), soil dissolved inorganic nitrogen (DIN) (c), soil dissolved organic nitrogen (DON) (d), soil dissolved total nitrogen (DTN) (e), and soil microbial biomass nitrogen (MBN) (f). The variables are categorized into different groups depending on the method type, ecosystem type, and frequency of FTC. Error bars represent 95% confidence intervals (CIs). If the 95% CI of $\overline{\ln R'}$ did not overlap with zero, a significant FTC effect was considered. The sample size for each category is shown next to the error bars
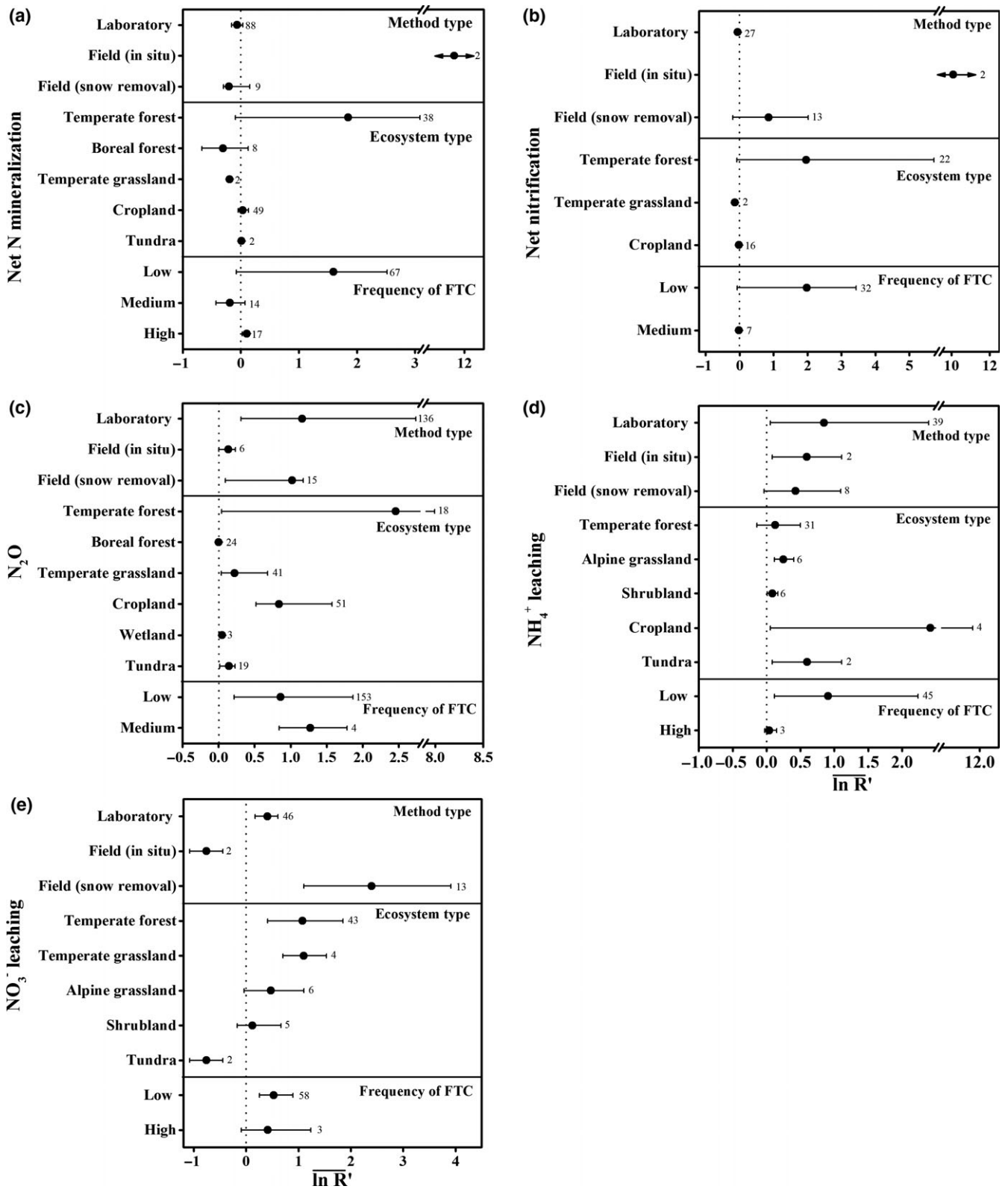
FIGURE 4 The mean effect size ($\overline{\ln R'}$) of freeze-thaw cycle (FTC) on soil net nitrogen (N) mineralization (a), soil net nitrification (b), N_2O emission (c), soil NH_4^+ leaching (d), and soil NO_3^- leaching (e). The variables are categorized into different groups depending on the method type, ecosystem type, and frequency of FTC. Error bars represent 95% confidence intervals (CIs). If the 95% CI of $\overline{\ln R'}$ did not overlap with zero, a significant FTC effect was considered. The sample size for each category is shown next to the error bars. Arrows stand for a larger 95% CI range

significantly positive in temperate forests, alpine forests, temperate grasslands, croplands, wetlands, and tundras (Figure 3b). The effect of FTC on DIN was significantly negative in temperate forest and temperate grassland and significantly positive in alpine forests and shrublands, but was not significant in croplands or tundras (Figure 3c). The response of DON to FTC was significantly positive in alpine forests, alpine grasslands, croplands, and wetlands, but was not significant in temperate forests, temperate grasslands, or tundras (Figure 3d). Under FTC treatment, DTN significantly decreased in temperate forests and increased in alpine forests, but did not significantly change in temperate grasslands, alpine grasslands, croplands, or tundras (Figure 3e). Soil MBN under FTC significantly reduced in alpine forests, alpine grasslands, and croplands, but did not change in temperate forests or tundras (Figure 3f).

Soil net N mineralization under FTC increased in temperate forests, but the effect was not statistically significant. The effect of FTC on net N mineralization was nonsignificant in boreal forests or croplands (Figure 4a). Soil net nitrification under FTC significantly decreased in temperate grasslands and croplands, but was not significantly affected by FTC in temperate forests (Figure 4b). Soil N_2O emission had more positive responses to FTC in temperate forests and croplands than temperate grasslands, wetlands, and tundras. But N_2O emission was not impacted by FTC in boreal forests (Figure 4c). The increase in soil NH_4^+ leaching under FTC was significant in alpine grasslands, shrublands, croplands, and tundras. But soil NH_4^+ leaching was not affected by FTC in temperate forests (Figure 4d). The increase in soil NO_3^- leaching under FTC was significant in temperate forests and temperate grasslands, but was not significant in alpine grasslands or shrublands (Figure 4e).

3.4 | Relationships between FTC patterns and the effect size of FTC on N pools and fluxes

The continuous randomized-effect model meta-analyses showed significantly positive correlations between the FTC duration and ln R' of FTC on TN, DIN, and NO_3^- leaching, and significantly negative correlations between the FTC duration and ln R' of FTC on DON (Tables 1, S4). The freeze duration had significantly positive correlations with ln R' of FTC on TN, DIN, NO_3^- leaching, net N mineralization, and net nitrification (Tables 1, S4). Significantly positive relationships were found between the thaw duration and ln R' of FTC on TN, DIN, NO_3^- leaching, net N mineralization, and net nitrification and significantly negative relationship was found between the thaw duration and ln R' of FTC on DTN (Tables 1, S4).

We found significantly positive correlations between the freeze temperature and ln R' of FTC on NO_3^- leaching, N_2O, and net nitrification and significantly negative correlations between the freeze temperature and ln R' of FTC on DIN, DON, and NH_4^+ (Table 1, Table S5).

The thaw temperature had significantly positive correlations with ln R' of FTC on DIN, DON, NO_3^-, and net nitrification and had significantly negative correlation with ln R' of FTC on DTN, NH_4^+

(Tables 1, S5). Significantly positive correlations were observed between the FTC amplitude and ln R' of FTC on DIN, DON, NO_3^-, and net nitrification and significantly negative correlations were observed between the FTC amplitude and ln R' of FTC on N_2O emission, net N mineralization (Tables 1, S5).

Our results showed that the FTC frequency was significantly positively correlated with ln R' of FTC on DIN and N_2O, and was significantly negatively correlated with ln R' of FTC on TN and DTN (Tables 1, S5). We further divided FTC frequency into three groups: low (≤ 5 times), medium (6~15 times), and high frequency (>15 times). Significantly positive effects of FTC on soil NH_4^+, NO_3^-, N_2O, NH_4^+ leaching, NO_3^- leaching and significantly negative effects of FTC on MBN were found at low frequency. FTC significantly increased soil NH_4^+, DIN, DON, N_2O at medium frequency. Significantly positive responses of soil NO_3^-, DIN, and net N mineralization to FTC and significantly negative response of DTN to FTC were observed at high frequency (Figures 3–4). In addition, soil moisture was significantly positively correlated with ln R' of FTC on NH_4^+, NO_3^- leaching, and N_2O, and was significantly negatively correlated with ln R' of FTC on DTN (Table 1).

4 | DISCUSSION

4.1 | Effects of FTC on soil N pools

4.1.1 | Soil NH_4^+ and NO_3^-

The increase in soil NH_4^+ subject to FTC was mainly from (i) the release from soil lattice; (ii) the release from inorganic and organic colloids; (iii) the release from dead soil microorganisms and the decrease in microbial immobilization; (iv) the increase in soil N mineralization rate; (v) the less root uptake in field studies; (vi) the decrease in other output fluxes of soil NH_4^+ besides immobilization and root uptake, including nitrification and leaching. FTC could disrupt soil structure, especially soil macroaggregates, which leads to the decrease in soil aggregate stability (Oztas & Fayetorbay, 2003; Six et al., 2004). The disruption of soil structure caused by FTC promotes the release of soil NH_4^+ from soil lattice and previously nonavailable NH_4^+ from organic and inorganic colloids (Freppaz, Williams, Edwards, Scalenghe, & Zanini, 2007) and the soil microorganisms killed during the FTC process could also be decomposed into NH_4^+ (Larsen et al., 2002; Yanai et al., 2004). Furthermore, the substrates released from the dead microorganisms could stimulate microbial activity and enhance the rate of N mineralization (Neilsen et al., 2001), thereby increasing soil NH_4^+. However, our results showed that FTC did not significantly impact net N mineralization, meaning the low possibility of the 4th mechanism. For the 5th mechanism, fine roots have been found to be susceptible to FTC processes (Campbell et al., 2014; Gaul et al., 2008; Reinmann & Templer, 2016) and fine root mortality was found to increase under FTC treatments based on a meta-analysis (Song et al., 2017). However, in laboratory incubation studies, while root uptake was not considered, soil NH_4^+ still increased under FTC. We found no

FTC patterns	N pools and fluxes	Q_T	Q_M	Q_E	Slope	p-value
Duration of FTC	DON	53.36	12.38	40.98	**−0.003**	0.000
	NO_3^- leaching	68.40	10.17	58.23	**0.001**	0.001
Duration of freeze	NO_3^- leaching	65.97	9.69	56.28	**0.004**	0.002
	Net N mineralization	67.18	11.27	55.91	**0.049**	0.001
	Net nitrification	35.70	14.57	21.13	**0.011**	0.000
Duration of thaw	DTN	106.11	5.90	100.21	**−0.018**	0.015
	NH_4^+	458.92	5.47	453.45	**0.008**	0.019
	NO_3^- leaching	71.60	15.33	56.26	**0.002**	0.000
	Net N mineralization	67.18	16.56	50.62	**0.039**	0.000
	Net nitrification	35.70	17.12	18.57	**0.028**	0.000
Temperature of freeze	DON	53.36	10.06	43.30	**−0.039**	0.002
	NH_4^+	495.44	18.02	477.42	**−0.016**	0.000
	NO_3^- leaching	69.20	9.22	59.98	**0.081**	0.002
	N_2O	130.34	4.52	125.82	**0.049**	0.034
	Net nitrification	30.33	4.00	26.34	**0.055**	0.046
Temperature of thaw	DTN	109.49	10.54	98.95	**−0.112**	0.001
	DON	43.85	7.86	35.98	**0.111**	0.005
	NH_4^+	492.35	18.11	474.23	**−0.033**	0.000
	NO_3^-	664.21	8.80	655.42	**0.016**	0.003
	Net nitrification	36.20	9.56	26.63	**0.049**	0.002
Amplitude of FTC	DON	43.85	8.97	34.88	**0.033**	0.003
	NO_3^-	630.67	7.92	622.75	**0.009**	0.005
	N_2O	117.56	5.36	112.20	**−0.055**	0.021
	Net N mineralization	101.07	24.20	76.87	**−0.031**	0.000
	Net nitrification	32.76	10.36	22.41	**0.041**	0.001
Frequency of FTC	DTN	112.49	12.85	99.64	**−0.015**	0.000
	N_2O	117.46	5.68	111.78	**0.180**	0.017
Soil moisture (% V)	DTN	68.98	22.55	46.43	**−0.017**	0.000
	NH_4^+	259.42	89.53	169.89	**0.019**	0.000
	NO_3^- leaching	17.53	4.44	13.09	**0.021**	0.035
	N_2O	48.37	10.23	38.14	**0.037**	0.001

TABLE 1 Relationships between the effect size (ln R') of freeze-thaw cycle (FTC) on nitrogen (N) pools and fluxes and the duration of FTC, the duration of freeze, the duration of thaw, temperature of freeze, temperature of thaw, amplitude of FTC, frequency of FTC, soil moisture. DON, soil dissolved organic N; DTN, soil dissolved total N (only significant relationships were presented and the complete presentation of the relationships was in Tables S4, S5). The bold values represent significant relationships between FTC patterns and N pools or fluxes

significant effect of FTC on NH_4^+ leaching or nitrification processes, point to the low possibility of the 6th mechanism. Therefore, we believe the first three mechanisms are most important to the increase in soil NH_4^+ and both the increase in soil NH_4^+ inputs and the decrease in microbial immobilization (an output flux of soil NH_4^+ hinted by decreased MBN) led to the increase in soil NH_4^+ under FTC processes.

Soil NO_3^- is mainly from nitrification processes and deposition and the output fluxes of soil NO_3^- include NO_3^- immobilization, denitrification, plant uptake, and leaching. We found the response of soil NO_3^- to FTC was positive, which can be explained by either the increase in the inputs or the decrease in the outputs or both. First, during the FTC period, although the release of NO_3^- from soil lattice and damaged microbial cells is also possible, a recent research using oxygen isotopes revealed that nearly all soil NO_3^- under FTC treatment was from the nitrification of soil NH_4^+ (Campbell et al., 2014).

We found a nonsignificant increase in net nitrification under FTC treatment, which could have contributed partly, but apparently not enough, to the increase in soil NO_3^-. Second, we found leaching of soil NO_3^- increased under FTC, which wound have caused opposite results on soil NO_3^-. In addition, although denitrification showed the trend of decrease based on the limited number of studies, the increase in N_2O emission suggested potential possibility of increased denitrification. Therefore, we believe the decreased microbial immobilization of NO_3^- may be an important reason of the enhanced soil NO_3^- content under FTC.

Soil NH_4^+ showed positive response to FTC in most ecosystems, except for the temperate forests. This was probably because nitrification as a loss pathway of NH_4^+, increased more in temperate forest ecosystems than in other ecosystems, consuming more NH_4^+. FTC significantly increased soil NO_3^- in all types of ecosystems except for alpine grasslands, which may be attributed to the less

effect of FTC on MBN in alpine grasslands compared to in other ecosystems, or the limited number of studies in alpine grasslands (28 observations from two studies).

The response of soil NH_4^+ was positively correlated with soil moisture, but was negatively correlated with the freeze temperature. The higher soil moisture and lower freeze temperature probably could induce larger destruction of soil lattices and soil microorganisms (Fitzhugh et al., 2001; Oztas & Fayetorbay, 2003; Zhou et al., 2011), thereby promoting the release of soil NH_4^+. The thaw temperature had opposite relationships with the response of soil NH_4^+ and NO_3^-, which was probably because that nitrifier activities accelerate with increasing thaw temperature, facilitating the transformation of NH_4^+ to NO_3^-. Soil NH_4^+ had positive response to FTC with low and medium frequencies, but had no response to FTC with high frequencies. Because the number of studies on high frequencies of FTC is low, more studies are needed to see if high frequencies would cause different responses of soil NH_4^+ from low and medium frequencies of FTC.

4.1.2 | DON and MBN

Our meta-analysis indicated that FTC had no significant effect on DON, but the response of DON to FTC was positive when only laboratory experiments were included. One of the main sources of soil DON is the disrupted microbial cells during the FTC process (Freppaz et al., 2007). More than half soil microbes were found to be dead in the first FTC in a previous laboratory study (Sawicka, Robador, Hubert, Jorgensen, & Bruchert, 2010), while in the field, due to the better acclimation of soil microbes to FTC (Grogan et al., 2004), soil microbes may be less affected. The unrealistically large temperature fluctuations in the laboratory incubation experiments and the unrealistic time of soil collection may also contribute to the difference between laboratory and field studies (Grogan et al., 2004; Henry, 2007; Koponen et al., 2006; Sharma, Szele, Schilling, Munch, & Schloter, 2006; Stenrød, Eklo, Charnay, & Benoit, 2005; Walker, Palmer, & Voordouw, 2006; Zhou et al., 2011). Soils sampled in different seasons have different properties, such as water content, root residues, litter, and snow cover depth. Soil microorganisms in different seasons have different tolerance to temperature (Schadt, Martin, Lipson, & Schmidt, 2003; Schmidt & Lipson, 2004), and microorganisms in soils sampled in seasons without FTC in laboratory studies may respond more severely to FTC than microorganisms in field studies. Therefore, laboratory studies should take these factors into account, including realistic temperature fluctuations, realistic time of soil collection, and proper soil moisture conditions.

The duration of FTC had significantly negative relationship with ln R' of DON, mainly due to the reduced releases of DON from dead soil microbes and disruptive soil aggregates with time and the more decomposition of DON with time. In contrast, soil DON was positively correlated with the thaw temperature and the amplitude of FTC. Because the increase in these two factors did not increase microbial death and decrease the response of net N mineralization to FTC, the increase in DON with increasing thaw temperature and

amplitude of FTC might be related to the changes of soil aggregates and should be further explored.

In our meta-analysis, FTC significantly decreased soil MBN in laboratory incubation experiments, but significantly increased soil MBN in the in situ studies or had no effect on soil MBN in the field with snow removal. The possible reasons for the discrepancy were as follows: (i) the limited number of field experiments (only two observations for the in situ study, only four observations for the snow removal study); (ii) the unrealistically large temperature fluctuations in the laboratory studies; (iii) the unrealistic time of soil collection for laboratory incubation as discussed above; and (iv) the quick changes of soil temperature in laboratory studies due to the small soil volumes used in the laboratory studies (Henry, 2007). The quick changes of soil temperature rarely occur in the fields when snowpack and plant litter are present. Because soil microbes are the drivers of N cycling, their increase or decrease directly affects N pools and fluxes and therefore should be carefully studied in future FTC researches. In addition to MBN, the responses of microbial community composition to FTC should also be explored more to better understand FTC effects on soil N cycling.

Soil MBN had no response to FTC in tundra or alpine grassland ecosystems, but had significantly negative response to FTC in croplands. Higher microbial resistance to FTC stress was often observed in alpine and arctic tundra ecosystems relative to other ecosystems (Grogan et al., 2004; Larsen et al., 2002; Lipson et al., 2000), because soil microbes are adapted to FTC stress and can quickly recover using the microbial necromass and the increased available soil nutrients after FTC in alpine and tundra ecosystems. Therefore, soil microbes in temperate regions may be more affected by future climate changes with more FTC events than those in arctic regions, which could have profound effects on the feedbacks of soil biogeochemical cycling to global climate changes.

4.1.3 | DTN and TN

For different ecosystem types and different patterns of FTC, the response of DTN generally agreed with the response of DIN and DON to FTC. The negative relationships between DTN and the duration of thaw, the temperature of thaw, and the frequency of FTC were mainly because that these FTC patterns exhibited significantly positive correlations with the losses of N_2O emission and NO_3^- leaching, leading to the decrease in DTN. In addition, the negative correlation between soil moisture and ln R' of DTN also reflected the important effect of soil water content on the losses of DTN. For laboratory incubation studies, although both DIN and DON increased under FTC, DTN did not change, which further suggested the limitations of laboratory studies.

Our results showed that FTC significantly decreased soil TN although there were only a few available studies, which can be partially explained by the significant increases in N losses via N_2O emission and NO_3^- leaching under FTC. These increased N losses play an important role in ecosystem productivity and environmental pollutions. For example, increased N losses could cause environmental

problems, such as aquatic eutrophication, greenhouse effects. (Ravishankara et al., 2009) and could lead to the decline of net primary production in N-limited ecosystems (Henry, 2007).

4.2 | Effects of FTC on N fluxes

4.2.1 | Net N mineralization and nitrification

Different from our expectation, neither net N mineralization nor nitrification showed significant responses to FTC, although they showed an increasing trend, especially when the duration of freeze and thaw got longer (indicated by the positive relationships between the duration of FTC and ln R' of net N mineralization and nitrification). When we further looked at different study methods and different ecosystems, the results also showed nonsignificant responses. We believe the reason was mainly that although the decreased soil microbes would cause decreased gross mineralization and nitrification, the decreased microbial immobilization of NH_4^+ and NO_3^- counterbalanced the effects, resulting in nonsignificant changes in the net processes under FTC.

4.2.2 | N2O and NO emissions

Soil N_2O emission is mainly from soil nitrification and denitrification processes (Groffman et al., 2009), while recent studies found that soil denitrification was the dominant process of soil N_2O production during FTC processes (Cui et al., 2016; Ludwig, Wolf, & Teepe, 2004; Mørkved, Dorsch, Henriksen, & Bakken, 2006; Risk, Snider, & Wagner-Riddle, 2013; Sharma et al., 2006; Teepe, Vor, Beese, & Ludwig, 2004; Wagner-Riddle, Hu, Van Bochove, & Jayasundara, 2008). Denitrification is an anaerobic process and is regulated by the availability of C and N, oxygen, soil moisture, soil temperature, and other parameters related to FTC (Risk et al., 2013; Wagner-Riddle et al., 2008). The increases of soil NO_3^- and soil nutrients were all beneficial for denitrifiers (Cui et al., 2016; Mørkved et al., 2006). More importantly, in frozen soil, the surface of soil particles is covered by the frozen water in the form of ice layers, which could form an anaerobic environment (Teepe, Brumme, & Beese, 2001), thereby triggering the production of N_2O in unfrozen microsites inside frozen soil particles during the freezing period. A vigorous N_2O emission has often been observed after thawing, which might be caused by (i) the damage of diffusion barriers after thawing (Matzner & Borken, 2008); (ii) the higher soil moisture during the thawing period in the anaerobic environment; and (iii) the strong acclimation of denitrifiers to the changing environment (Smith, Wagner-Riddle, & Dunfield, 2010) and the quick recovery during thawing (Müller, Kammann, Ottow, & Jager, 2003). In fact, we found a significantly positive correlation between ln R' of N_2O emission and soil moisture. However, no significant effect of FTC on soil denitrification was observed in our results. While the number of studies on soil denitrification was limited (19 observations), we believe it could be possibly because that N_2 production during the denitrification process was not measured well, resulting in the underestimation of denitrification rates.

The response of N_2O emission to FTC was relatively higher in croplands and temperate forest ecosystems compared to that in other ecosystems, which could be explained by the higher increases in NO_3^- in croplands and temperate forest ecosystems.

NO emission from soils subject to FTC significantly reduced by 25.8% according to the limited number of studies (Figure 5). NO is mainly produced during nitrification and could be further oxidized to N_2O in a more anaerobic environment (Goldberg, Muhr, Borken, & Gebauer, 2008; Yao et al., 2010). We believe that the more
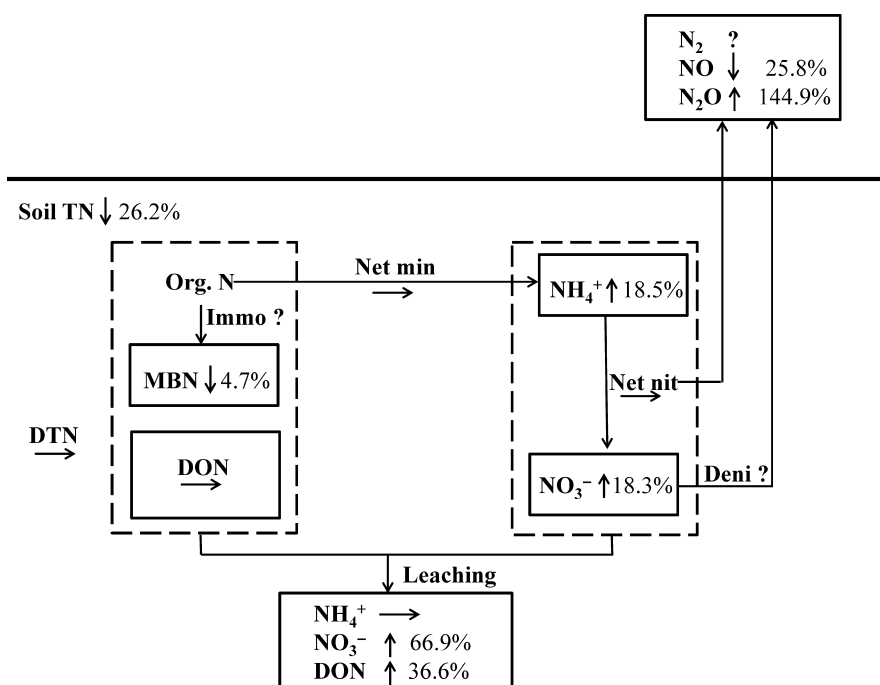
FIGURE 5 Responses of terrestrial ecosystem N pools and biogeochemical processes to the freeze-thaw cycle (FTC). TN, soil total nitrogen; Org. N, soil organic nitrogen; DON, soil dissolved organic nitrogen; DTN, soil dissolved total nitrogen; MBN, microbial biomass nitrogen; Net min, net N mineralization; Immo, immobilization; Net nit, net nitrification; Deni, denitrification. The numbers and ↑ represent increases by FTC. The numbers and ↓ represent decreases by FTC. →, no significant change; ?, there are currently not enough studies

anaerobic microsites under FTC caused the oxidation and the reduction of NO. The losses of gaseous N during FTC processes might be one of the major causes of low N use efficiency in croplands (Davidson & Janssens, 2006) and an important issue in natural ecosystems as well.

4.2.3 | N leaching losses

It is difficult for soil NH_4^+ to move due to the adsorption of soil particles (Macdonald et al., 2002), which was the reason for the less responses of NH_4^+ leaching compared to soil NO_3^- leaching under FTC. Our results also denoted that FTC significantly enhanced DON leaching by 36.6%, due to the increase in soil DON under FTC. Soil DON was found to replace NO_3^- as the main form of N losses in the initial period of FTC in a previous study (Freppaz et al., 2007), pointing to the importance of DON leaching.

NO_3^- leaching increased under FTC in temperate forests and temperate grassland ecosystems, but decreased in tundra ecosystems. Tundra and arctic ecosystems may have lower soil moisture and N content, which reduced the risk of N leaching. We found that NO_3^- leaching was significantly positively correlated with soil moisture and the freeze temperature, probably ascribed to the effect of water availability on leaching and the lower NO_3^- content at lower freeze temperature, consistent with the findings in tundra ecosystems.

The interaction effects were not discussed in this study because we focused on the general patterns across different studies while the number of studies under the interaction effects was limited. For example, for DIN, out of the twelve interactions between "ecosystem type" (six levels) and "method" (two levels), laboratory incubation studies in alpine forests ($n = 18$), alpine shrublands ($n = 6$), and field experiments in temperate forests ($n = 8$) showed positive responses while laboratory incubation studies in temperate forests ($n = 4$) and temperate grasslands ($n = 2$) showed negative responses and laboratory incubation studies in croplands ($n = 23$) and tundra ($n = 3$) showed no response to FTC; the rest interactions had no observation so far. Therefore, more studies are needed to understand the variations of the FTC effects under the interactions of different factors of the experiment.

In conclusion, our meta-analysis results showed that FTC can significantly increase soil NH_4^+, NO_3^-, NO_3^- leaching, DON leaching, as well as N_2O emission, and significantly decrease soil TN and MBN. Because net N mineralization or nitrification rates did not change under FTC, the effects of FTC on soil N pools were mainly caused by the abrupt release from soil lattice and inorganic and organic colloids from the damage of soil aggregates. Although soil microbes were slightly killed during the processes of FTC, they could quickly recover and their activities may not change. If global climate warming increases the duration of FTC, the temperature of freeze, the amplitude of freeze, and the frequency of FTC, the release of inorganic N, the losses of N leaching, N_2O emissions, and even mineralization and nitrification processes are all expected to increase, aggravating N limitation in the early spring period, especially for the N-limited ecosystems. To accurately predict the responses of soil N pools and fluxes to FTC, future experiments should focus on field studies or simulations with realistic changes of temperature and proper sample collection time in laboratory studies.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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Graphical Abstract

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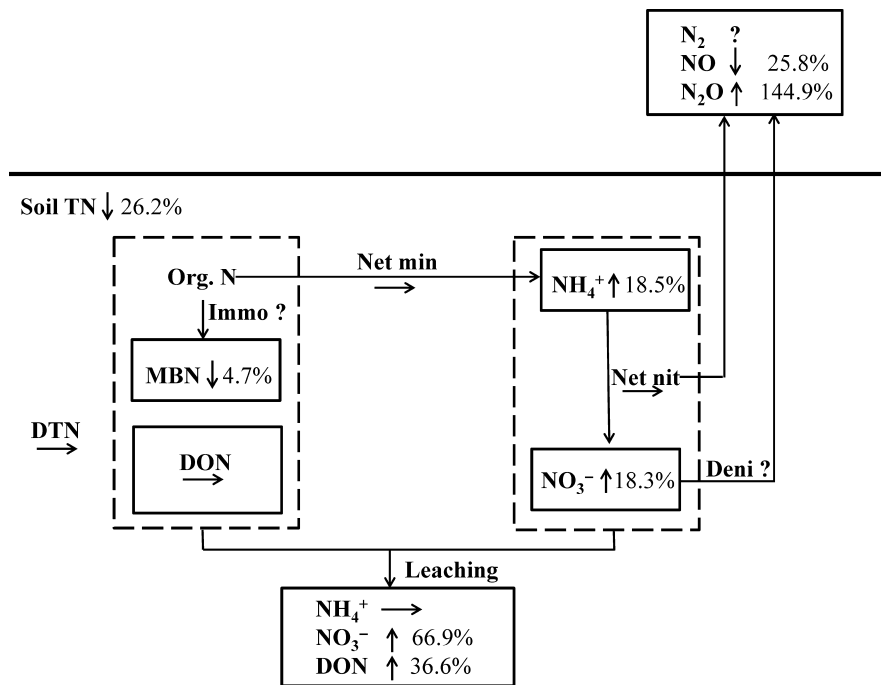
1. Freeze-thaw cycle significantly increased inorganic N, NO_3^- leaching, and N_2O emission, but significantly decreased soil total N and microbial biomass nitrogen. 2. Temperate and cropland ecosystems with relatively high soil nutrient contents were more responsive to FTC than alpine and arctic tundra ecosystems with rapid microbial acclimation. 3. Altered FTC patterns (such as increased duration of FTC, temperature of freeze, amplitude of freeze, and frequency of FTC) due to global climate warming would enhance the release of inorganic N and the losses of N via leaching and N_2O emissions.