

Do freeze-thaw events enhance C and N losses from soils of different ecosystems? A review

E. MATZNER & W. BORKEN

Soil Ecology, University of Bayreuth, D-95440 Bayreuth, Germany

Summary

Freezing and thawing of soils may affect the turnover of soil organic matter and thus the losses of C and N from soils. Here we review the literature with special focus on: (i) the mechanisms involved, (ii) the effects of freezing temperature and frequency, (iii) the differences between arable soils and soils under natural vegetation, and (iv) the hypothesis that freeze-thaw events lead to significant C and N losses from soils at the annual scale. Changes in microbial biomass and populations, root turnover and soil structure might explain increased gaseous and solute fluxes of C and N following freeze-thaw events, but these mechanisms have seldom been addressed in detail. Effects of freeze-thaw events appear to increase with colder frost temperatures below 0°C, but a threshold value for specific soils and processes cannot be defined. The pool of C and N susceptible to freeze-thaw events is rather limited, as indicated by decreasing losses with short-term repeated events. Elevated nitrate losses from soils under alpine and/or arctic and forest vegetation occurred only in the year following exceptional soil frost, with greatest reported losses of about 13 kg N ha⁻¹. Nitrate losses are more likely caused by reduced root uptake rather than by increased N net mineralization. N₂O emissions from forest soils often increased after thawing, but this lasted only for a relatively short time (days to 1–2 months), with the greatest reported cumulative N₂O emissions of about 2 kg N₂O-N ha⁻¹. The emissions of N₂O after freeze-thaw events were in some cases substantially greater from arable soils than from forest soils. Thus, freeze-thaw events might induce gaseous and/or solute losses of N from soils that are relevant at the annual time scale. While a burst of CO₂ after thawing of frozen soils is often found, there is strong evidence that, at the annual time scale, freeze-thaw cycles either have little effect or will even reduce soil C losses as compared with unfrozen conditions. On the contrary, a milder winter climate with fewer periods of soil frost may result in greater losses of C from soils that are presently influenced by extended frost periods.

Definition

We realise that the ‘true’ freezing point of soil is at or close to 0°C, depending on whether there is a significant amount of solute in the soil water. In this paper, we refer to studies in which soils and related materials have been cooled, naturally or artificially, well below this temperature. For the sake of clarity and simplicity, we refer to the temperature to which the soil has been cooled or exposed either as the frost temperature or the freezing temperature. Likewise a decrease in freezing or frost temperature means that conditions became colder, an increase means that they became warmer.

Introduction

Soils play a major role in the global C and N cycle because they represent much larger reservoirs of organic C and N than the terrestrial plants. Given the projected tendency for an increase in temperature, the effects of climatic changes on microbial soil processes and the sequestration of C and N have become a matter of intensive debate (Davidson & Janssens, 2006). Soil processes at low temperatures may be of special importance in that context, because temperature sensitivity is greater at lower temperatures (Fang & Moncrieff, 2001; Mikan *et al.*, 2002). Soil respiration and N mineralization in the winter period is far from being negligible at the annual scale (Grogan *et al.*, 2004; Campbell *et al.*, 2005; Kielland *et al.*, 2006; Monson *et al.*, 2006) and both processes continue also at soil temperatures below 0°C in unfrozen water films (Edwards & Cresser, 1972; Panikov *et al.*, 2006).

Correspondence: E. Matzner. E-mail: egbert.matzner@uni-bayreuth.de
Received 20 June 2007; revised version accepted 29 October 2007

Freezing and thawing of soils occur regularly in higher latitudes. The intensity and frequency of soil frost is mainly dependent on regional climatic conditions and the thickness of an insulating snow cover. With global climate change less snow cover will enhance soil frost in some regions, while in other regions the general warming may decrease frost intensity and frequency as well as the duration of soil frost. That freeze-thaw events influence the turnover of C and N in soil is long known in principle and a large body of literature has developed on that subject over the past decade. A burst of CO₂ or N₂O emissions from thawing soils is often observed (Nielsen *et al.*, 2001; Teepe & Ludwig, 2004) and in some cases more than 50% of the annual emission of N₂O is found in the thaw period (Papen & Butterbach-Bahl, 1999; Teepe *et al.*, 2000). The occurrence of soil frost is also related to the increase in nitrate leaching from forest ecosystems (Fitzhugh *et al.*, 2003; Watmough *et al.*, 2004; Callesen *et al.*, 2007). Such observations raise the question of the relevance of freeze-thaw events for C and N losses from soils. Answers to this question are not easily found because many studies are short-term, laboratory-based, have no flux measurements or control treatments and sometimes only focus on the thaw period rather than on the frost period. Furthermore, unrealistic freezing regimes as well as sampling dates of soil and disturbance of soil samples are major drawbacks in many freeze-thaw studies (Henry, 2007). The review on effects of freeze-thaw events by Henry (2007) focused on methodological aspects. In this review we explicitly address the following aspects:

- mechanisms involved in changing rates of C and N turnover during freeze-thaw events;
- effects of frost temperature and frequency of freeze-thaw events on C and N turnover;
- differences between fertilized arable/grassland soils and soils under natural vegetation (alpine and/or arctic and forest); and
- the hypothesis that freeze-thaw cycles cause additional gaseous and solute losses that significantly influence the C and N budget at the annual scale.

We concentrate on temperate and boreal soils under different land uses, but do not consider permafrost and wetland soils explicitly.

Mechanisms involved in C and N turnover under freeze-thaw events

Generally, three mechanisms are considered to explain the often increased fluxes of C and N after thawing of frozen soil: (i) release from microbial biomass, (ii) death of roots, and (iii) changes in soil structure.

Release from microbial biomass and shifts in microbial populations

One of the few studies with detailed investigations on the role of microbial biomass was by Herrmann & Witter (2002) on an

arable soil. After labelling the microbial biomass with ¹⁴C glucose they reported that 65% of the CO₂ flush after thawing was due to decomposition of the microbial necromass. Investigating the causes for N₂O emissions after thawing of a frozen arable soil, Sharma *et al.* (2006) were able to trace the effect to increased activity of denitrifiers. In another study on N₂O emissions from arable soils, Dörsch *et al.* (2004) observed a decrease of the microbial biomass after freezing. The microbial biomass quickly recovered after thawing, indicating the use of microbial necromass by the surviving community. Similarly, DeLuca *et al.* (1992) attributed the increase of N mineralization after thawing of an arable soil to the use of amino-N from the lysis of microorganisms. By using phospholipid fatty acid patterns, Feng *et al.* (2007) report decreasing biomass of fungi after severe freezing (−15°C) while the bacterial biomass remained unaffected.

In contrast to arable soils, results from alpine and tundra soils often revealed no freeze-thaw effects on microbial biomass (Lipson & Monson, 1998; Lipson *et al.*, 2000; Grogan *et al.*, 2004). A similar finding is reported by Koponen *et al.* (2006) for arable soils in Finland. In these soils microbial populations appear to be adapted to frost stress. This was confirmed for alpine soils beneath meadow and larch by Freppaz *et al.* (2006), who also reported that the microbial biomass decreased under frost at −9°C in alpine soils beneath fir and alder. The reasons for the differences between sites remained unclear.

Root turnover

The death of roots due to soil frost and their subsequent decomposition might be a factor influencing C and N fluxes in the thaw period in soils under permanent vegetation. Tierney *et al.* (2001) reported increased fine root (< 1 mm diameter) mortality after frost in two forest sites. The additional root litter after the frost was estimated at 300 kg ha^{−1}, which is roughly equivalent to 150 kg C ha^{−1}. Assuming that 50% of this C is mineralized in the first growing season after the frost, the extra CO₂ flux would be 75 kg C ha^{−1}, which is only 2–3% of the expected annual rate of soil respiration (Groffman *et al.*, 2006). However, a short-term flush of CO₂ can be caused by the decomposition of soluble cell constituents after root death. The contribution of root turnover to N mineralization will depend on the C/N ratio of roots and the net mineralization rate. Tierney *et al.* (2001) calculated an additional N input to the soil by root necromass after frost of about 5 kg N ha^{−1}. This input was causally related to the observed increase in N fluxes with soil solutions in the post-thaw period. Assuming that 50% of the 5 kg N ha^{−1} in root necromass is mineralized in the next growing season, this amounts to only 2.5 kg N ha^{−1}. Thus, it is rather unlikely that C and N fluxes after thawing of forest soils will significantly increase due to rapid decomposition of fine roots. This conclusion is supported by Groffman *et al.*

(2006), who reported no increase in soil respiration after frost events at the same sites that were used by Tierney *et al.* (2001). On the contrary, if we assume a permanently greater root litter input, the C pool of the soil might even increase due to frequent freeze-thaw events.

Changes in soil structure

The hypothesis that changes in soil structure are involved in freeze-thaw effects is based on two mechanisms: (i) the physical disruption of macro- and microaggregates by frost might expose previously inaccessible substrates to subsequent mineralization at faster rates; and (ii) slower rates of diffusion and advection under frozen conditions might cause bursts of gaseous emissions after thawing. Although often proposed, little direct evidence for the effects of changes in physical structure on C and N fluxes is available. The observed burst of N₂O emissions after frost was greater in small than in large macro-aggregates, which was attributed to the greater water content of smaller aggregates, which enhanced aggregate destruction (van Bochove *et al.*, 2000). Indeed, the effect of frost on soil structure increases with the water content of the soil prior to frost, as shown in the case of N₂O emissions from a forest soil (Öquist *et al.*, 2004). However, Öquist *et al.* (2004) attributed their findings to the effect of water content on the formation of anoxic microsites rather than to aggregate destruction. Morkved *et al.* (2006) concluded that the N₂O pulses were due to increased availability of C sources after frost that fuelled denitrifiers and oxygen consumption.

From studies on N₂O and CO₂ emissions from arable soils, Teepe *et al.* (2001) as well as Elberling & Brandt (2003) and Koponen *et al.* (2004) emphasize the role of diffusion and advection barriers in the creation of gaseous emission bursts after thawing. They concluded that the observed burst of emissions was due to production of gases in unfrozen microsites during soil frost, with subsequent emission after the thaw destroying the diffusion barriers. The production of N₂O can also continue in deeper, unfrozen soil horizons, with subsequent accumulation underneath the frozen layer.

Depending on the frost temperature, a proportion of soil water adhering to soil particles remains unfrozen (Edwards & Cresser, 1972). As a consequence of ice formation, the unfrozen solutions will have greater concentrations of substrates and enzymes and, together with slow diffusion rates of O₂, trigger the production of N₂O.

Summarizing our knowledge on mechanisms, most studies remain speculative and only a few have investigated mechanisms in detail. The relevance of single mechanisms differs not only between field and laboratory studies but also in their importance under different environments. It is likely that several mechanisms act simultaneously and that the importance of individual mechanisms changes with space and time, for example due to differences in freezing history, initial water content, conductivity, and freezing temperature.

Effects of freezing temperature on C and N turnover

Biological processes continue at soil temperatures below 0°C and colder freezing temperatures may reduce the turnover rate of C and N according to general temperature sensitivity of the processes involved. Frost effects on microorganisms might be most important for C and N turnover because of their role as catalysts of C and N mineralization and, after lysis, their role as substrates. Systematic studies on freezing-temperature effects are rare and comparisons between different studies are difficult to make because the depth of soil temperature measurements and soil conditions differ between field studies, responses seem process specific, and different experimental methods were used. Some often-cited studies report frost effects on pure bacterial cultures from arable soils. Skogland *et al.* (1988) found a CO₂ burst after freezing a culture at -7°C, which was attributed to the decay of bacterial necromass, whereas Morley *et al.* (1983) found no effect of frost on *Pseudomonas* cultures at -9°C, but considerable mortality at -27°C. Given the fact that the conditions for microorganisms in a frozen soil are very different from pure cultures and considering the small number of culturable microorganisms in soils, results from pure cultures with bacteria are of little help in predicting *in situ* frost effects on soil microbial populations.

There is evidence that frost decreased microbial biomass in arable soils at -5°C (Herrmann & Witter, 2002; Dörsch *et al.*, 2004). On the contrary, in alpine and arctic soils, frost at -4 to -5°C had no effect on microbial biomass (Lipson & Monson, 1998; Lipson *et al.*, 2000, 2002; Grogan *et al.*, 2004). Neilsen *et al.* (2001) reported a substantial increase in CO₂ emissions, after thawing, from forest floor samples frozen at -13°C in comparison with frost at -3°C. Respiration in the -3°C treatment was also greater than in the non-frozen controls. Studies with soil columns from a Norway spruce site at freezing temperatures of -3, -8 and -13°C resulted in a decrease of N mineralization after thawing in relation to an unfrozen control (Hentschel *et al.*, 2008). The -8°C and -13°C treatment had the strongest effects but the -8°C treatment did not differ from that at -13°C. In the same experiment, CO₂ production increased after thawing relative to the control, with the -13°C treatment causing the largest effect (Goldberg *et al.*, 2008).

Based on the literature, a general conclusion seems that C and N turnover after thawing increase with colder frost temperatures. However, a threshold value for specific soils and processes cannot be defined. It is also an open question if adaptation of microbial communities to lower temperatures has occurred in some soils or soil horizons while not in others. Even after strong frost, microbial biomass seems to recover quickly (Stenberg *et al.*, 1998; Pesaro *et al.*, 2003), while frost effects on slow growing specific microbial communities (e.g. nitrifiers, methanotrophs) need more attention in future work.

Effects of freeze-thaw frequency

The relevance of freeze-thaw events for C and N turnover at longer time scales will depend on their frequency. Herrmann & Witter (2002) exposed arable soils to up to 20 short-term freeze-thaw cycles during 40 days. The observed effects on respiration and N mineralization were greatest in the first cycles and decreased in later cycles. Similar findings were reported from other studies on arable (Priemé & Christensen, 2001), tundra (Schimel & Klein, 1996) and forest soils (Kurganova & Tipe, 2003; Goldberg *et al.*, 2008; Hentschel *et al.*, 2008). Emissions of N₂O from arable soils also decreased in a series of freeze-thaw cycles (Koponen & Martikainen, 2004). The pool of C and N susceptible to freeze-thaw events was found to be rather limited if these occurred within short periods. The magnitude of the replenishment of the pools when there is a longer break between freeze-thaw events remains an open question.

Relevance of freeze-thaw events for C and N losses from soils

Do freeze-thaw events significantly influence the losses of C and N from soils when annual time scales are considered? We consider as significant changes fluxes that exceed the estimated error of the annual flux measurement. The answer to this question will depend on the relation of the process rates and fluxes during the freeze-thaw cycle in comparison with an unfrozen control (Figure 1). Respiration and N mineralization continue at temperatures well below 0°C – presumably in unfrozen water films, but freezing reduces the rate of microbial soil processes as compared with the unfrozen soil. The duration of the frost will determine the overall effect of the freeze-thaw cycle. If the total amount of

C or N emitted or leached during and after thawing is larger than the amount trapped in the frost period, a net loss will result and *vice versa*.

Many laboratory studies did not use a control treatment, but instead interpreted the temporal dynamics of processes in soil samples under frozen and subsequently thawed conditions. This may cause misinterpretation of the results because the cumulative effect of a freeze-thaw cycle on the sink and source strength of the soil can only be evaluated in relation to a control treatment. Thus, the result will also depend on the conditions of the control, raising the question of how to define the control. For example, if a control treatment in a laboratory experiment is kept at permanently +1°C, the manipulated freeze-thaw treatment might result in a faster cumulative process rate (or net loss of C and N) after the entire experiment (Figure 1a), while relative to a control at +5°C the effect might not be observed or, even the opposite might be found (Figure 1b). Some studies have used very high temperatures for controls (Wang & Bettany, 1993, +23°C; Feng *et al.*, 2007, +17°C) and in fact, their results on CO₂ emissions differ from those of other authors. The temperature of control treatments thereby determines the results of freeze-thaw experiments, and emphasizes the critical role of the experimental design. Hence, the temperature of controls during the freezing and thawing periods of the manipulated samples should be carefully considered in the evaluation of published experiments as well as in the design of future laboratory experiments. For laboratory experiments, we suggest that the temperature of the unfrozen control should parallel the one of the treatment until 0°C has been reached. During thawing the temperatures of control and treatment should be as similar as possible. The control conditions will also influence the outcome of experimental field studies where the

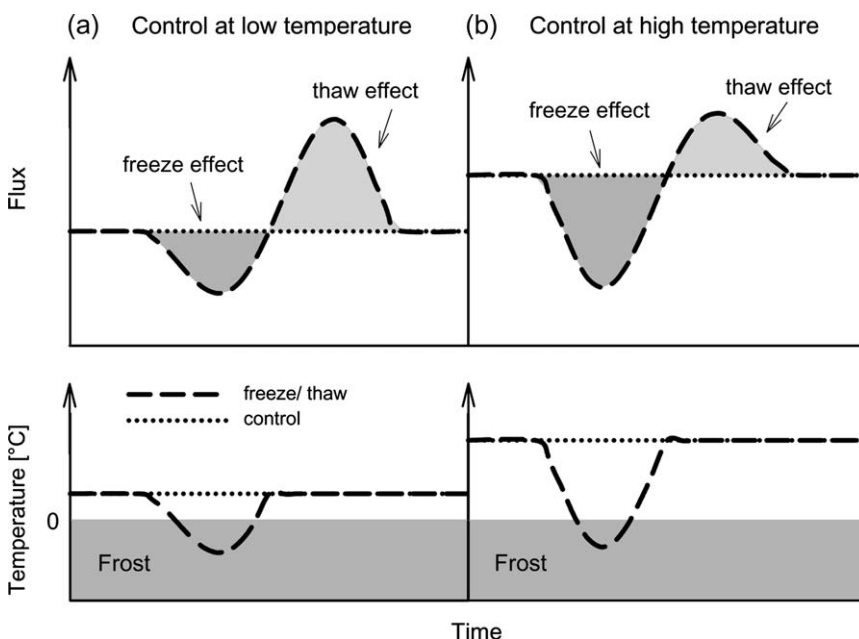


Figure 1 Temperature of the controls determines the observed effects of freeze-thaw events on C and N fluxes. Fluxes less than control are equivalent to relative sequestration, fluxes exceeding control are equivalent to additional losses. (a) control at low temperature causing a cumulative net loss relative to the control; (b) control at high temperature causing a cumulative net sequestration relative to the control.

temperature and water content of the control plots cannot be influenced, thereby causing variations of the treatment effects in different years.

Freeze-thaw effects and nitrogen losses from soils

Nitrogen losses from soils as a consequence of freeze-thaw might occur in solute form (NH_4^+ , NO_3^- , dissolved organic N) or as gaseous emissions (NO , N_2O , N_2). In unfertilized soils, the net mineralization of N is an important precondition for potential N losses. Thus, we will first discuss findings on N net mineralization and then compile data from flux measurements. An increase in N net mineralization does not necessarily lead to additional N losses from the soil, as N uptake by roots or later re-immobilization by microbes can occur. However, increased net mineralization points to potential risks of N losses.

Nitrogen net mineralization

DeLuca *et al.* (1992) found an up to twofold increase of N mineralization in arable soils for about 20 days after thawing. Nitrogen mineralization also increased two- to threefold in formerly frozen arable soils (Herrmann & Witter, 2002), but again the effect was rather short-lived (40 days). Findings from soils under natural vegetation are contradictory. Neilsen *et al.* (2001) observed an increase of N mineralization in Oa layers subjected to frost at -13°C , whereas frost at -3°C had no effect. In the Oe and A horizons, mineralization was unaffected by any frost treatment. Effects on the soil were larger under maple than under birch, the reasons remaining unclear. Increased N mineralization as an effect of soil frost was also reported for an arctic soil by Grogan *et al.* (2004) and for different alpine soils by Freppaz *et al.* (2006). For alpine soils, Schmidt & Lipson (2004) proposed a cycle of N immobilization under soil frost by specific microbial populations adapted to temperatures $< 0^\circ\text{C}$, with subsequent N release from their necromass after thawing.

In contrast to these findings of increased N mineralization, Groffman *et al.* (2001) reported *in situ* N mineralization to be unaffected in a birch and a maple site after snow cover removal and moderate soil frost (about -1°C at 10 cm depth, Hardy *et al.*, 2001). Schimel & Klein (1996) showed a threefold increase of N mineralization in an alder-poplar tundra soil after the first freeze-thaw cycle at -5°C , while no change was observed in the following cycles. In three other tundra soils, cumulative N mineralization was less than the control after three freeze-thaw cycles. The reduced N mineralization corresponds to the findings of Larsen *et al.* (2002), who reported smaller amounts of extractable NH_4 in freeze-thaw (-4°C) experiments with arctic soil mesocosms relative to the unfrozen controls or to the permanently frozen samples. In a snow cover manipulation experiment in an arctic soil, Schimel *et al.* (2004) found greater rates of N mineralization in winter under deep snow cover and moderate soil frost (-5°C) than at sites

with little snow cover and severe soil frost (-20°C) – similar to the findings of Hentschel *et al.* (2008) and Sulkava & Huhta (2003) for forest soils.

Generally, freeze-thaw events seem to increase N net mineralization in arable soils after thawing, which is probably due to the small C/N ratios and high pH of arable soils. However, there is no convincing evidence for a general increase in N mineralization in soils under natural vegetation. The increase in mineralization, if observed, seems to be rather small in relation to the expected annual mineralization rates.

Solute-N fluxes under field conditions

Out of eight studies on solute-N fluxes affected by freeze-thaw events, seven report increased fluxes after exceptional soil frost events (Table 1). The available data only cover soils under arctic and/or alpine and forest vegetation; no information can be found for solute fluxes as affected by freeze-thaw events for arable/grassland soils. Mitchell *et al.* (1996) concluded from empirical studies in NE America forested watersheds that an increase of nitrate fluxes in runoff and of NO_3^- concentrations in soil solution was due to an unusually cold preceding winter. The additional losses of NO_3^- in the following year were about $1.4\text{--}2.8 \text{ kg NO}_3^-\text{-N ha}^{-1} \text{ year}^{-1}$. The intensity of soil frost also explained the inter-annual variation of NO_3^- concentrations in Hubbard Brook streams, but the relation of frost to NO_3^- fluxes was weak, the latter being mainly dominated by hydrological conditions (Fitzhugh *et al.*, 2003). The relevance of soil frost for NO_3^- concentrations in runoff at Hubbard Brook was, however, questioned by a modelling exercise that simulated the NO_3^- fluxes without taking freeze-thaw effects into account (Hong *et al.*, 2005). In a study on 16 Canadian watersheds, Watmough *et al.* (2004) found soil frost to be a driver of large NO_3^- concentrations in wetland-influenced streams, whereas the effect of frost did not show up in upland-draining streams. In a Norway spruce site in Germany subjected to large N deposition rates, NO_3^- fluxes with seepage increased after an exceptional soil frost in the following year by about $13 \text{ kg N ha}^{-1} \text{ year}^{-1}$ (Callesen *et al.*, 2007).

To our knowledge, only three studies have reported *in situ* soil solution N concentrations after experimental manipulation of snow cover and soil frost, thereby providing direct evidence of freeze-thaw effects on N leaching. Boutin & Robitaille (1995) induced severe soil frost of -5°C to 40-cm depth in a sugar maple site in Canada after snow removal. The control site was unfrozen at 10-cm depth. The increase of soil solution N concentration at 30-cm depth was substantial in the following vegetation period, but no fluxes of soil solution N were calculated. Nitrate fluxes in runoff from an alpine soil increased substantially after exposure to deep soil frost (-11°C) in relation to sites of moderate frost (-5°C) (Brooks *et al.*, 1998). As a result, $11.4 \text{ kg N ha}^{-1}$ were lost from the deep frost site during snowmelt relative to 2.7 kg N ha^{-1} from the moderate frost site. In their snow removal experiment, Fitzhugh *et al.*

Table 1 Cumulative N losses from soils as affected by freeze-thaw events.

Ecosystem	N ₂ O losses	Solute losses	Minimum soil temperature/depth	Soil temperature after thawing/depth	Type of study	Length of soil freezing period at specific soil depth	References
Arable + fertilized grassland	Increase		NA	+4°C/NA	Field	NA	Christensen & Tiedje (1990)
	Increase		−5°C/5 cm	0°C/5 cm	Field	3 months, 5 cm	Dörsch <i>et al.</i> (2004)
	Increase		NA	NA	Field	NA	Flessa <i>et al.</i> (1995)
	Increase		NA	NA	Field	NA	Müller <i>et al.</i> (2003)
	Increase		−1°C/5 cm	+7°C/5 cm	Field	NA	Nyborg <i>et al.</i> (1997)
	Increase		NA	NA	Field	NA	Regina <i>et al.</i> (2004)
	Increase		NA	NA	Field	NA	Syväsalo <i>et al.</i> (2004)
	Increase		−1.5 to −15°C	+4°C	Laboratory	5 days–2 weeks	Koponen & Martikäinen (2004)
	Increase		−5°C	+10°C	Laboratory	6 days	Kurganova <i>et al.</i> (2004)
	Increase		−8°C	+8°C	Laboratory	5 days	Ludwig <i>et al.</i> (2004)
	Increase		−20°C	+20°C	Laboratory	2 days	Müller <i>et al.</i> (2002)
	Increase		−15°C	+4°C	Laboratory	3 weeks	Priemé & Christensen (2001)
	Increase	Increase	−11°C/0 cm	0°C/0 cm	Field	5 months, surface	Brooks <i>et al.</i> (1998)
Alpine/arctic	Increase		−4°C	+8°C	Laboratory	15 days	Grogan <i>et al.</i> (2004)
Forest		Increase	−5°C/40 cm	NA	Field	5 months, 10 cm	Boutin & Robitaille (1995)
		Increase	−3°C/15 cm	NA	Field	3 months, 15 cm	Callesen <i>et al.</i> (2007)
	Increase		−2°C/10 cm	0°C/10 cm	Field	NA	Goodroad & Keeney (1984)
		Increase	−1°C/10 cm	NA	Field	5 months, 10 cm	Fitzhugh <i>et al.</i> (2001)
		Increase	NA	NA	Field	NA	Fitzhugh <i>et al.</i> (2003)
		Increase	NA	NA	Field	NA	Mitchell <i>et al.</i> (1996)
	Increase		−5°C/5 cm	+5°C/5 cm	Field	3 month, 5 cm	Papen & Butterbach-Bahl (1999)
		Increase	NA	NA	Field	NA	Watmough <i>et al.</i> (2004)
	Decrease		−3 to −13°C	+5°C	Laboratory	14 days	Goldberg <i>et al.</i> (2008)
		Decrease	−3 to −13°C	+5°C	Laboratory	14 days	Hentschel <i>et al.</i> (2008)
	Increase		−5°C	+10°C	Laboratory	6 days	Kurganova <i>et al.</i> (2004)

NA = not available.

(2001) observed an increase of total N fluxes in forest floor percolates from 16 (control) to 29 (treatment, −1°C at 10 cm depth) kg N ha^{−1} year^{−1} in the sugar maple stand and from 16 (control) to 54 (treatment) kg N ha^{−1} year^{−1} in the yellow birch stand. Despite noticeable effects in the forest floor percolates, the total N fluxes from the deeper soil depth (Bs horizon) were not affected at the yellow birch site and increased by about 3 kg N ha^{−1} year^{−1} at the sugar maple site.

Summarizing, several studies have shown indirect evidence for the potential of soil frost to trigger N leaching from soils under natural vegetation and direct evidence is also available from field experiments. Effects were not observed in every case and tree species, because soil conditions and the status of N saturation are likely to have a strong influence. Elevated N fluxes from deeper soil depths occurred only in the year after soil frost and are obviously due to reduced nitrate uptake by roots rather than to increased N net mineralization. No information is available for arable soils, but we expect only minor effects of freeze-thaw events, given the short-lived flush in N mineralization (see above) and the overwhelming effect of management and fertilization on nitrate leaching from arable soils.

Relevance of gaseous versus solute N-losses

To our knowledge, the only study in which emissions of gaseous N compounds and solute fluxes were measured simultaneously during a freeze-thaw event is that of Grogan *et al.* (2004) in an arctic tundra soil. Thus, the evaluation of the relevance of gaseous versus solute losses has to be based on the comparison of different studies. Here, we concentrate on studies that provide fluxes on an area basis (Table 1).

Out of 17 papers, 16 report increasing N₂O emissions following freeze-thaw events in all types of ecosystems. The absolute amounts of additional N₂O emissions from arable soils after freeze-thaw events differ by several orders of magnitude. However, the rates are often much greater than under natural vegetation. A range of 1–300 g N ha^{−1} emitted in a few days is reported from laboratory studies on arable soils (Koponen & Martikäinen, 2004; Kurganova *et al.*, 2004; Koponen *et al.*, 2006). In field studies, the variation was also very great. The extra N₂O flux after freeze-thaw was about 0.7 kg N ha^{−1} in the study of Dörsch *et al.* (2004). Much larger rates of emissions were reported by Flessa *et al.* (1995) and Regina *et al.*

Table 2 Cumulative C losses from soils as affected by freeze-thaw events

Ecosystem	CO ₂ pulse after thawing	Effect on cumulative C losses	Minimum soil temperature/depth	Soil temperature after thawing/depth	Type of study	Length of freezing period	References
<i>Arable + fertilized grassland</i>	Yes	Increase	-5°C/5 cm	0°C/5 cm	Field	3 months, 5 cm	Dörsch <i>et al.</i> (2004)
	Yes	Decrease	-15°C	+17°C	Laboratory	1 day	Feng <i>et al.</i> (2007)
	Yes	No	-2°C to -5°C	+5°C	Laboratory	6 hours	Herrmann & Witter (2002)
	Yes	Increase	-5°C	+10°C	Laboratory	6 days	Kurganova <i>et al.</i> (2004)
	Yes	Increase	-15°C	+4°C	Laboratory	3 weeks	Priemé & Christensen (2001)
<i>Alpine/arctic</i>	No	Decrease	-10°C	+23°C	Laboratory	1 day	Wang & Bettany (1993)
	Yes	NA	NA	0-5°C/2,5 cm	Field	NA	Elberling & Brandt (2003)
	Not studied	Decrease	-1.5°C/NA	NA	Field	NA	Monson <i>et al.</i> (2006)
	No	No	-4°C	+8°C	Laboratory	15 days	Grogan <i>et al.</i> (2004)
	No	Decrease	-4°C	+2°C	Laboratory	NA	Larsen <i>et al.</i> (2002)
<i>Forest</i>	Yes	No	-5°C	+5°C	Laboratory	5 days	Schimmel & Klein (1996)
	No	No	NA	NA	Field	NA	Groffman <i>et al.</i> (2006)
	Yes	No	-8°C/8 cm	NA	Field	2 month, 8 cm	Coxson & Parkinson (1987)
	Yes	NA	-3 to -13°C	+5°C	Laboratory	14 days	Goldberg <i>et al.</i> (2008)
	Yes	No	-3 to -13°C	+20 to +25°C	Laboratory	10 days	Neilsen <i>et al.</i> (2001)

NA = not available.

(2004), who attributed up to 46% of the annual rates (7–25 kg N₂O-N ha⁻¹ year⁻¹) to freeze-thaw events. Large emission rates from arable soils after thawing were also observed by Sylväsalo *et al.* (2004) and Nyborg *et al.* (1997).

In a laboratory column study with forest soils, cumulative N₂O emissions after thawing of a silty soil amounted to 1.5 kg N ha⁻¹, but were much less in a sandy soil (Teepe & Ludwig, 2004). In experiments with an arctic soil, the maximum rate of about 10 g N₂O-N ha⁻¹ day⁻¹ lasted for only a few days (Grogan *et al.*, 2004). In a field study in a White pine forest, N₂O emissions during and after thawing cumulated to 0.84 kg N ha⁻¹ (Goodroad & Keeney, 1984). In a Norway spruce forest, a severe soil frost might have been responsible for an extra N₂O emission of about 2.5 kg N ha⁻¹ after thawing (Papen & Butterbach-Bahl, 1999).

With regard to total gaseous N losses, the fluxes of NO and N₂ need to be taken into account, the ratio mainly depending on redox conditions and process rates (Davidson *et al.*, 2000). In some cases, NO emissions exceeded those of N₂O by far (Butterbach-Bahl *et al.*, 2002; Goldberg *et al.*, 2008), but NO has only rarely been measured in freeze-thaw events. The unknown in gaseous N emissions from soils remains N₂, because of the methodical problems associated with the measurement.

In summary, additional N emissions during and after thawing last only for a relatively short time (days to 1–2 months) but may reach substantial rates that are relevant for the overall N turnover in arable soils. In arable soils, the source of the emissions seems to be largely fertilizer N, given the quick response of fluxes after thawing. In forest soils, the greatest additional rates of

N₂O emissions after thawing were about 2 kg N ha⁻¹. The potential losses of gaseous N after freeze-thaw events from forest soils might thus be of the same order of magnitude as the potential additional nitrate losses. However, large unknowns exist for all ecosystems with respect to NO and N₂ emissions.

Freeze-thaw effects and carbon losses from soils

With respect to the C losses from soils, we will focus on CO₂ emissions. Only minor amounts of C are lost as dissolved organic carbon (DOC) in relation to CO₂ and emissions of CH₄ are only of relevance for wetland soils. Wang & Bettany (1993) report slightly increased amounts of extractable DOC after a freeze-thaw event in an arable soil, but an increase of DOC concentrations in soil solutions at deeper soil layers or in runoff as a potential effect of freeze-thaw events has not been reported yet. There is a lack of field studies on CO₂ emissions focusing on freeze-thaw effects and our knowledge on that topic is largely based on laboratory experiments, many of which have been conducted with arctic and/or alpine soils. Problems of interpretation result also from the fact that most studies concentrate on the thawing period and do not consider processes under frozen conditions (see also Figure 1).

In arable soils, three out of four studies report an increase in cumulative CO₂ emissions after freeze-thaw events and all studies found a pulse of CO₂ after thawing (Table 2). On the contrary, none of the nine studies on soils under natural vegetation documented an increase in cumulative CO₂ emissions, while five also report a pulse of CO₂ after thawing. Two studies under natural vegetation even report reduced cumulative

CO₂ emissions following freeze-thaw events. In a field study with arable soils, the extra emission of CO₂ after a freeze-thaw event was rather small (about 180 kg C ha⁻¹, Dörsch *et al.*, 2004). In another laboratory study with arable soils, the additional CO₂ losses as a result of thawing amounted to 0–130 kg C ha⁻¹ and the effect decreased with successive freeze-thaw cycles (Priemé & Christensen, 2001). Thus, the amounts of C lost additionally from arable soils represent only a small percentage (< 5%) of the annual soil respiration, even if the largest values are considered.

In a 6-year field study in an alpine ecosystem (Monson *et al.*, 2006), the lesser soil respiration in cold years (about 120 kg C ha⁻¹ year⁻¹) was equivalent to 21% of the mean cumulative ecosystem C sequestration. The CO₂ production after thawing of the frozen soil did not compensate for the reduction during the frost period. Elberling & Brandt (2003) reported an unpredicted burst of CO₂ after soil thawing in a tundra heathland, which they attributed to the release of trapped CO₂ from deeper soil layers. Overall the relevance of the burst was considered to be small, because 95% of the CO₂ emission in the growing season was explained only by soil temperature and moisture fluctuations. In two hardwood forests, soil respiration was unaffected by experimentally induced freeze-thaw events (Groffman *et al.*, 2006).

A number of laboratory studies with soils under natural vegetation also reported little or negative effects of freeze-thaw events on CO₂ production from soils and seem to confirm the hypothesis that soil frost rather results in less C losses than under unfrozen soil conditions (Coxson & Parkinson, 1987; Larsen *et al.*, 2002; Grogan *et al.*, 2004). In their study on tundra soils, a net loss of CO₂ due to three freeze-thaw cycles was only observed in the wet meadow soil at a generally low level of CO₂ emissions (Schimel & Clein, 1996). In three other soils with forest and tussock vegetation, the effect was not observed and CO₂ losses were not different between control and treated soils after three freeze-thaw cycles.

In summary, while a short-lived burst of CO₂ after thawing is often found, there is strong evidence that freeze-thaw events will either have little effect on soil C losses at an annual time scale or will be counteracted by less C losses from frozen soil than from unfrozen soil. Substantial additional C losses as a consequence of freeze-thaw cycles find little support, especially for soils under natural vegetation.

Overall conclusions and research needs

Our initial hypothesis, that freeze-thaw cycles cause additional gaseous and solute losses relevant to soil C and N budgets at the annual time scale was only confirmed for N. Here, substantial gaseous N losses, presumably from fertilizer N, have been documented for arable soils. For soils under natural vegetation, gaseous N losses triggered by freeze-thaw events have also been documented, but at a generally lesser rate than in arable soils.

These losses, together with increased and substantial solute N losses, document the potential of freeze-thaw events to cause N losses relevant at the annual time scale in all types of ecosystems.

Freeze-thaw events obviously only cause small additional losses of C from arable soils, while for soils under natural vegetation no effect, or even less soil respiration – relative to a control soil – was found. Thus, a milder winter climate with fewer periods of soil frost may result in losses of organic C from soils under natural vegetation that are presently influenced by extended periods of soil frost.

Table 3 provides a conceptual overview of the parameters that seem relevant for cumulative C and N losses after freeze-thaw events and that explain some of the contradictions in the literature. As factors triggering enhanced C and N losses, we see the degree of soil aggregation, the soil water content at freezing, the size of the labile C and N pools and the frost temperature, while extended periods of frost and frequent freeze-thaw events seem to reduce losses. In the case of N, the fertilization input to arable soils and the degree of N saturation of natural ecosystems will also influence the potential loss of N after freeze-thaw events.

The state of knowledge on the effects of freeze-thaw events on C and N fluxes in soils is characterized by the fact that experimental studies often follow only single parameters, short-term laboratory experiments dominate, and comprehensive analysis of gaseous and solute fluxes under field conditions are extremely rare. Many studies have only focussed on processes during the thaw period. This, together with the limited number of studies from different soils, conducted under varying conditions, neither allows quantification of the factors given in Table 3, nor ranking of their relevance. Thus, Table 3 also points to substantial research needs.

Future experimental work should cover different soils and vegetation types, with a focus on field studies, and simultaneously measure N and C fluxes in both gaseous and solute forms. Special emphasis should be given to the temperatures of the controls. The mechanisms that change soil process rates

Table 3 Parameters potentially influencing the cumulative effect of freeze-thaw events on C and N losses from soils.

Increase of	Trend for cumulative CO ₂ and losses	Trend for cumulative gaseous and solute N losses
Aggregation	Increase	Increase
Soil water content at freezing	Increase	Increase
Duration of frost period	Decrease	Decrease
Pool of labile C and N	Increase	Increase
Frost temperature	Decrease	Decrease
Water fluxes after thawing		Increase
N-fertilization of arable soils		Increase
Status of N saturation of natural ecosystems		Increase

during freeze-thaw events, for example the dynamics of reactive soil pools and of specific microbial populations need to be explicitly addressed such that a better understanding of contradictory findings can be achieved. Interdisciplinary approaches are needed that comprise expertise from soil physics, microbiology, plant ecology and soil ecology. Such studies will not only improve our understanding of the functioning of soils in general, but will also allow a better assessment of the effects of future climatic changes on soil processes.

Acknowledgements

We would like to thank the Deutsche Forschungsgemeinschaft (DFG) for financial support.

References

- van Bochove, E., Prévost, D. & Pelletier, F. 2000. Effects of freeze-thaw and soil structure on nitrous oxide produced in a clay soil. *Soil Science Society of America Journal*, **64**, 1638–1643.
- Boutin, R. & Robitaille, G. 1995. Increased soil nitrate losses under mature sugar maple trees affected by experimentally induced deep frost. *Canadian Journal of Forest Research*, **25**, 588–602.
- Brooks, P.D., Williams, M.W. & Schmidt, S.K. 1998. Inorganic nitrogen and microbial biomass dynamics before and during spring snowmelt. *Biogeochemistry*, **43**, 1–15.
- Butterbach-Bahl, K., Breuer, L., Gasche, R., Willibald, G. & Papen, H. 2002. Exchange of trace gases between soils and the atmosphere in Scots pine forest ecosystems of the northeastern German lowlands 1. Fluxes of N₂O, NO/NO₂ and CH₄ at forest sites with different N-deposition. *Forest Ecology and Management*, **167**, 123–134.
- Callesen, I., Borken, W., Kalbitz, K. & Matzner, E. 2007. Long-term development of nitrogen fluxes in a coniferous ecosystem: does soil frost trigger nitrate leaching? *Journal of Plant Nutrition and Soil Science*, **170**, 189–196.
- Campbell, J.L., Mitchell, M.J., Groffman, P.M., Christenson, L.M. & Hardy, J.P. 2005. Winter in northeastern North America: a critical period for ecological processes. *Frontiers in Ecology and the Environment*, **3**, 314–322.
- Christensen, S. & Tiedje, J.M. 1990. Brief and vigorous N₂O production by soil at spring thaw. *Journal of Soil Science*, **41**, 1–4.
- Coxson, D.S. & Parkinson, D. 1987. Winter respiratory activity in Aspen woodland forest floor litter and soils. *Soil Biology & Biochemistry*, **19**, 49–59.
- Davidson, E.A. & Janssens, I.A. 2006. Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. *Nature*, **440**, 165–173.
- Davidson, E.A., Keller, M., Erickson, H.E., Verchot, L.V. & Veldkamp, E. 2000. Testing a conceptual model of soil emissions of nitrous and nitric oxides. *BioScience*, **50**, 667–680.
- DeLuca, T.H., Keeney, D.R. & McCarty, G.W. 1992. Effect of freeze-thaw events on mineralization of soil nitrogen. *Biology and Fertility of Soils*, **14**, 116–120.
- Dörsch, P., Palojärvi, A. & Mommertz, S. 2004. Overwinter greenhouse gas fluxes in two contrasting agricultural habitats. *Nutrient Cycling in Agroecosystems*, **70**, 117–133.
- Edwards, A.C. & Cresser, M.S. 1972. Freezing and its effect on chemical and biological properties of soil. *Advances in Soil Science*, **18**, 59–79.
- Elberling, B. & Brandt, K.K. 2003. Uncoupling of microbial CO₂ production and release in frozen soil and its implications for field studies of arctic C cycling. *Soil Biology & Biochemistry*, **35**, 263–272.
- Fang, C. & Moncrieff, J.B. 2001. The dependence of soil CO₂ efflux on temperature. *Soil Biology & Biochemistry*, **33**, 155–165.
- Feng, X., Nielsen, L.L. & Simpson, J. 2007. Response of soil organic matter and microorganisms to freeze/thaw cycles. *Soil Biology & Biochemistry*, **39**, 2027–2037.
- Fitzhugh, R.D., Driscoll, C.T., Groffman, P.M., Tierney, G.L., Fahey, T.J. & Hardy, J.P. 2001. Effects of soil freezing disturbance on soil solution nitrogen, phosphorus, and carbon chemistry in a northern hardwood ecosystem. *Biogeochemistry*, **56**, 215–238.
- Fitzhugh, R.D., Driscoll, C.T., Groffman, P.M., Tierney, G.L., Fahey, T.J. & Hardy, J.P. 2003. Soil freezing and the acid-base chemistry of soil solutions in a northern hardwood forest. *Soil Science Society of America Journal*, **67**, 1897–1908.
- Flessa, H., Dörsch, P. & Beese, F. 1995. Seasonal variation of N₂O and CH₄ fluxes in differently managed arable soils in southern Germany. *Journal of Geophysical Research*, **100**, 23115–23124.
- Freppaz, M., Williams, B.L., Edwards, A.C., Scalenghe, R. & Zanini, E. 2006. Simulating soil freeze/thaw cycles typical of winter alpine conditions: implications for N and P availability. *Applied Soil Ecology*, **35**, 247–255.
- Goldberg, S., Muhr, J., Borken, W. & Gebauer, G. 2008. Fluxes of climate-relevant trace gases between a Norway spruce, forest soil and the atmosphere during freezing/thawing cycles in mesocosms. *Journal of Plant Nutrition and Soil Science*, in press.
- Goodroad, L.L. & Keeney, D.R. 1984. Nitrous oxide emission from soils during thawing. *Canadian Journal of Soil Science*, **64**, 187–194.
- Groffman, P.M., Driscoll, C.T., Fahey, T.J., Hardy, J.P., Fitzhugh, R.D. & Tierney, G.L. 2001. Effects of mild winter freezing on soil nitrogen and carbon dynamics in a northern hardwood forest. *Biogeochemistry*, **56**, 191–213.
- Groffman, P.M., Hardy, J.P., Driscoll, C.T. & Fahey, T.J. 2006. Snow depth, soil freezing, and fluxes of carbon dioxide, nitrous oxide and methane in a northern hardwood forest. *Global Change Biology*, **12**, 1748–1760.
- Grogan, P., Michelsen, A., Ambus, P. & Jonasson, S. 2004. Freeze-thaw regime effects on carbon and nitrogen dynamics in sub-arctic heath tundra mesocosms. *Soil Biology & Biochemistry*, **36**, 641–654.
- Hardy, J.P., Groffman, P.M., Fitzhugh, R.D., Henry, K.S., Welman, A.T., Demers, J.D. *et al.* 2001. Snow depth manipulation and its influence on soil frost and water dynamics in a northern hardwood forest. *Biogeochemistry*, **56**, 151–174.
- Henry, H.A.L. 2007. Soil freeze-thaw cycle experiments: trends methodological weaknesses and suggested improvements. *Soil Biology & Biochemistry*, **39**, 977–986.
- Hentschel, K., Borken, W. & Matzner, E. 2008. Leaching losses of nitrogen and dissolved organic matter following repeated frost/thaw events in a forest soil. *Journal of Plant Nutrition and Soil Science*, in press.
- Herrmann, A. & Witter, E. 2002. Sources of C and N contributing to the flush in mineralization upon freeze-thaw cycles in soils. *Soil Biology & Biochemistry*, **34**, 1495–1505.

- Hong, B., Swaney, D.P., Woodbury, P.B. & Weinstein, D.A. 2005. Long-term nitrate export pattern from Hubbard Brook Watershed 6 driven by climatic variation. *Water, Air and Soil Pollution*, **160**, 293–326.
- Kielland, K., Olson, K., Ruess, R.W. & Boone, R.D. 2006. Contribution of winter processes to soil nitrogen flux in taiga forest ecosystems. *Biogeochemistry*, **81**, 349–360.
- Koponen, H.T. & Martikainen, P.J. 2004. Soil water content and freezing temperature affect freeze-thaw related N₂O production in organic soil. *Nutrition Cycling in Agroecosystems*, **69**, 213–219.
- Koponen, H.T., Flöjt, L. & Martikainen, P.J. 2004. Nitrous oxide emissions from agricultural soils at low temperatures: a laboratory microcosm study. *Soil Biology & Biochemistry*, **36**, 757–766.
- Koponen, H., Jaakkola, T., Keinänen-Toivola, M.M., Kaipainen, S., Tuomainen, J., Servomaa, K. *et al.* 2006. Microbial communities, biomass, and activities in soils as affected by freeze thaw cycles. *Soil Biology & Biochemistry*, **38**, 1861–1871.
- Kurganova, I.N. & Tipe, P. 2003. The effects of freezing-thawing processes on soil respiration activity. *Eurasian Soil Science*, **36**, 976–985.
- Kurganova, I.N., Teepe, R. & Lopes de Gerenyu, V.O. 2004. The dynamics of N₂O emissions from arable and forest soils under alternating freeze-thaw conditions. *Eurasian Soil Science*, **37**, 1219–1228.
- Larsen, K.S., Jonasson, S. & Michelsen, A. 2002. Repeated freeze-thaw cycles and their effects on biological processes in two arctic ecosystem types. *Applied Soil Ecology*, **21**, 187–195.
- Lipson, D.A. & Monson, R.K. 1998. Plant-microbe competition for soil amino acids in the alpine tundra: effects of freeze-thaw and dry-rewet events. *Oecologia*, **113**, 406–414.
- Lipson, D.A., Schmidt, S.K. & Monson, R.K. 2000. Carbon availability and temperature control the post-snowmelt decline in alpine soil microbial biomass. *Soil Biology & Biochemistry*, **32**, 441–448.
- Lipson, D.A., Schadt, C.W. & Schmidt, S.K. 2002. Changes in soil microbial community structure and function in an alpine dry meadow following spring snow melt. *Microbial Ecology*, **43**, 307–314.
- Ludwig, B., Wolf, I. & Teepe, R. 2004. Contribution of nitrification and denitrification to the emission of N₂O in a freeze-thaw event in an agricultural soil. *Journal of Plant Nutrition and Soil Science*, **167**, 678–684.
- Mikan, C.J. & Schimel, J.P. & Doyle, A.P. 2002. Temperature controls of microbial respiration in arctic tundra soils above and below freezing. *Soil Biology & Biochemistry*, **34**, 1785–1795.
- Mitchell, M.J., Driscoll, C.T., Kahl, J.S., Likens, G.E., Murdoch, P.S. & Pardo, L.H. 1996. Climatic control of nitrate loss from forested watersheds in the northeast United States. *Environmental Science & Technology*, **30**, 2609–2612.
- Monson, R.K., Lipson, D.L., Burns, S.P., Turnipseed, A.A., Delany, A.C., Williams, M.W. *et al.* 2006. Winter forest soil respiration controlled by climate and microbial community composition. *Nature*, **439**, 711–714.
- Morkved, P.T., Dörsch, P., Henriksen, T.M. & Bakken, L.R. 2006. N₂O emissions and product ratios of nitrification and denitrification as affected by freezing and thawing. *Soil Biology & Biochemistry*, **38**, 3411–3420.
- Morley, C.R., Trofymow, J.A., Coleman, D.C. & Cambardella, C. 1983. Effects of freeze-thaw stress on bacterial populations in soil microcosms. *Microbial Ecology*, **9**, 329–340.
- Müller, C., Martin, M., Stevens, R.J., Laughlin, R.J., Kammann, C., Ottow, J.C.G. *et al.* 2002. Processes leading to N₂O emissions in grassland soil during freezing and thawing. *Soil Biology & Biochemistry*, **34**, 1325–1331.
- Müller, C., Kammann, C., Ottow, J.C.G. & Jäger, H.J. 2003. Nitrous oxide emission from frozen grassland soil and during thawing periods. *Journal of Plant Nutrition and Soil Science*, **166**, 46–53.
- Neilsen, C.B., Groffman, P.M., Hamburg, S.P., Driscoll, C.T., Fahey, T.J. & Hardy, J.P. 2001. Freezing effects on carbon and nitrogen cycling in northern hardwood forest soils. *Soil Science Society of America Journal*, **65**, 1723–1730.
- Nyborg, M., Laidlaw, J.W., Solberg, E.D. & Malhi, S.S. 1997. Denitrification and nitrous oxide emissions from a Black Chernozemic soil during spring thaw in Alberta. *Canadian Journal of Soil Science*, **77**, 153–160.
- Öquist, M.G., Nilsson, M., Sörensson, F., Kasimir-Klemetsson, A., Persson, T., Weslien, P. *et al.* 2004. Nitrous oxide production in a forest soil at low temperatures - processes and environmental controls. *FEMS Microbiology Ecology*, **49**, 371–378.
- Panikov, N.S., Flanagan, P.W., Oechel, W.C., Mastepanov, M.A. & Christensen, T.R. 2006. Microbial activity in soils frozen to below –39°C. *Soil Biology & Biochemistry*, **38**, 785–794.
- Papen, H. & Butterbach-Bahl, K. 1999. A 3-year continuous record of nitrogen trace gas fluxes from untreated and limed soil of a N-saturated spruce and beech forest ecosystem in Germany. 1. N₂O emission. *Journal of Geophysical Research*, **104**, 18487–18503.
- Pesaro, M., Widmer, F., Nicollier, G. & Zeyer, J. 2003. Effects of freeze-thaw stress during soil storage on microbial communities and methadation degradation. *Soil Biology & Biochemistry*, **35**, 1049–1061.
- Priemé, A. & Christensen, S. 2001. Natural perturbations, drying-wetting and freezing-thawing cycles, and the emission of nitrous oxide, carbon dioxide and methane from farmed organic soils. *Soil Biology & Biochemistry*, **33**, 2083–2091.
- Regina, K., Syväsalö, E., Hannukkala, A. & Esala, M. 2004. Fluxes of N₂O from farmed peat soils in Finland. *European Journal of Soil Science*, **55**, 591–600.
- Schimel, J.P. & Klein, J.S. 1996. Microbial response to freeze-thaw cycles in tundra and taiga soils. *Soil Biology & Biochemistry*, **28**, 1061–1066.
- Schimel, J.P., Bilbrough, C. & Welker, J.M. 2004. Increased snow depth affects microbial activity and nitrogen mineralization in two Arctic tundra communities. *Soil Biology & Biochemistry*, **36**, 217–227.
- Schmidt, S.K. & Lipson, D.A. 2004. Microbial growth under the snow: implications for nutrient and allelochemical availability in temperate soils. *Plant and Soil*, **259**, 1–7.
- Sharma, S., Szele, Z., Schilling, R., Munch, J.C. & Schlöter, M. 2006. Influence of freeze-thaw stress on the structure and function of microbial communities and denitrifying populations in soil. *Applied and Environmental Microbiology*, **72**, 2148–2154.
- Skogland, T., Lomeland, S. & Goksoyr, J. 1988. Respiratory burst after freezing and thawing of soil: experiments with soil bacteria. *Soil Biology & Biochemistry*, **20**, 851–856.
- Stenberg, B., Johansson, M., Pell, M., Sjö Dahl-Svensson, K., Stenström, J. & Torstensson, L. 1998. Microbial biomass and activities in soil as affected by frozen and cold storage. *Soil Biology & Biochemistry*, **30**, 393–402.

- Sulkava, P. & Huhta, V. 2003. Effects of hard frost and freeze-thaw cycles on decomposer communities and N mineralisation in boreal forest soil. *Applied Soil Ecology*, **22**, 225–239.
- Syväsalo, E., Regina, K., Pihlatie, M. & Esala, M. 2004. Emissions of nitrous oxide from boreal agricultural clay and loamy sand soils. *Nutrient Cycling in Agroecosystems*, **69**, 155–165.
- Teepe, R. & Ludwig, B. 2004. Variability of CO₂ and N₂O emissions during freeze-thaw cycles: results of model experiments on undisturbed forest-soil cores. *Journal of Plant Nutrition and Soil Science*, **167**, 153–159.
- Teepe, R., Brumme, R. & Beese, F. 2000. Nitrous oxide emissions from frozen soils under agricultural, fallow and forest land. *Soil Biology & Biochemistry*, **32**, 1807–1810.
- Teepe, R., Brumme, R. & Beese, F. 2001. Nitrous oxide emissions from soil during freezing and thawing periods. *Soil Biology & Biochemistry*, **33**, 1269–1275.
- Tierney, G., Fahey, T.J., Groffman, P.M., Hardy, J.P., Fitzhugh, R.D. & Driscoll, C.T. 2001. Soil freezing alters fine root dynamics in a northern hardwood forest. *Biogeochemistry*, **56**, 175–190.
- Wang, F.L. & Bettany, R.R. 1993. Influence of freeze-thaw and flooding on the loss of solute organic carbon and carbon dioxide from soil. *Journal of Environmental Quality*, **22**, 709–714.
- Watmough, S.A., Eimers, M.C., Aherne, J. & Dillon, P.J. 2004. Climate effects on stream nitrate concentrations at 16 forested catchments in South Central Ontario. *Environmental Science & Technology*, **38**, 2382–2388.