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TOPICAL REVIEW

A review of the importance of mineral nitrogen cycling in the plant-soil-microbe system of permafrost-affected soils—changing the paradigm

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Abstract

The paradigm that permafrost-affected soils show restricted mineral nitrogen (N) cycling in favor of organic N compounds is based on the observation that net N mineralization rates in these cold climates are negligible. However, we find here that this perception is wrong. By synthesizing published data on N cycling in the plant-soil-microbe system of permafrost ecosystems we show that gross ammonification and nitrification rates in active layers were of similar magnitude and showed a similar dependence on soil organic carbon (C) and total N concentrations as observed in temperate and tropical systems. Moreover, high protein depolymerization rates and only marginal effects of C:N stoichiometry on gross N turnover provided little evidence for N limitation. Instead, the rather short period when soils are not frozen is the single main factor limiting N turnover. High gross rates of mineral N cycling are thus facilitated by released protection of organic matter in active layers with nitrification gaining particular importance in N-rich soils, such as organic soils without vegetation. Our finding that permafrost-affected soils show vigorous N cycling activity is confirmed by the rich functional microbial community which can be found both in active and permafrost layers. The high rates of N cycling and soil N availability are supported by biological N fixation, while atmospheric N deposition in the Arctic still is marginal except for fire-affected areas. In line with high soil mineral N production, recent plant physiological research indicates a higher importance of mineral plant N nutrition than previously thought. Our synthesis shows that mineral N production and turnover rates in active layers of permafrost-affected soils do not generally differ from those observed in temperate or tropical soils. We therefore suggest to adjust the permafrost N cycle paradigm, assigning a generally important role to mineral N cycling. This new paradigm suggests larger permafrost N climate feedbacks than assumed previously.

1. Introduction—are permafrost-affected soils characterized by organic N cycling only?

Over the last decades, the nitrogen (N) cycle of permafrost-affected soils has been perceived to involve mostly organic N compounds with little importance of the production and turnover of mineral N (see figure 1(a)). This paradigm has been established since the landmark paper of Schimel and Bennett (2004) which postulates that in cold ecosystems—in contrast to temperate and tropical systems—a strong temperature limitation of protein depolymerization limits the availability of dissolved organic N compounds (DON, e.g. amino acids). Consequently, DON would be exposed to intense plant-microbe competition so that microbes would remain N-limited but not carbon-limited, which forces them to use the assimilated N for growth rather than for performing ammonification (Regina *et al* 1996, Schimel and Bennett 2004). Because ammonification is the first step of mineral N production and turnover, it provides the substrate for other mineral N cycling processes in the plant-soil-microbe system. Hence, impaired ammonification suppresses the entire mineral N cycle, i.e. subsequent nitrification (the microbial oxidation of ammonium (NH_4^+) and ammonia (NH_3) to nitrite (NO_2^-) and nitrate (NO_3^-)), denitrification (the reduction of NO_3^- or NO_2^- to gaseous N such as the greenhouse gas (GHG) nitrous oxide (N_2O) and inert dinitrogen gas (N_2)), and microbial immobilization of NH_4^+ and NO_3^- (figure 1(a)). Such a 'short' N-conserving cycle is characterized by high N limitation and efficient ecosystem N retention, but by little importance of mineral N turnover such as ammonification and nitrification that leads to gaseous or hydrological N losses from the ecosystem. A wide range of studies has emphasized the prevalence of organic N over mineral N forms to be characteristic for N-limited arctic ecosystems (Kielland 1995, Jones and Kielland 2002, Weintraub and Schimel 2005, Weedon *et al* 2012, Wild *et al* 2018). As a consequence of the predominance of organic N cycling there would be no significant emissions of microbially produced N_2O and other N gases, while carbon (C) mineralization still facilitates production and emission of the GHGs carbon dioxide (CO_2) and methane (CH_4).

Based on these assumptions, together with the large amounts of C stored in permafrost-affected soils (Hugelius *et al* 2014), permafrost research interests during the last decades have focused on climate feedbacks of the C cycle via emission of the GHGs CO_2 and CH_4. The total gaseous C release resulting from permafrost degradation might be as high as 92 ± 17 Pg C within this century, with an estimated contribution by CO_2 and CH_4 of 97.7% and 2.3%, respectively (Schuur *et al* 2015). These GHG emissions from warming permafrost-affected soils are thought to be large enough to significantly contribute to the global temperature increase in the 21st century (Schuur *et al* 2013). What is less known, however, is that permafrost-affected soils store and potentially emit large—though less well constrained—amounts of N (Harden *et al* 2012, Ramm *et al* 2020, Voigt *et al* 2020). Particularly N_2O is of major concern as it is a long-lived GHG with a 100 year global warming potential exceeding those of CO_2 and CH_4 by 265 and 9.5 times, respectively (IPCC 2014). Furthermore, N_2O plays a dominant role in stratospheric ozone destruction in the 21st century (Ravishankara *et al* 2009).

Permafrost N pools and associated soil N turnover processes (protein depolymerization, ammonification, nitrification) started to receive increasing attention only 1–2 decades ago. A current search in the Web of Science and Scopus (March 2021) indicated several hundred studies on 'permafrost/arctic C turnover', around 100 studies on 'permafrost/arctic N turnover' and only about ten studies on 'permafrost/arctic gross N turnover'. Thus, the general observation that understanding of N cycling is lagging behind relevant work on C cycling for decades (Schlesinger 2009) is particularly true for permafrost-affected ecosystems. Deficits in knowledge on ecosystem N cycling compared to C cycling persist—not only concerning permafrost ecosystems—due to a range of severe methodological problems to accurately quantify the complex N cycling in the plant-soil-microbe system (Groffman *et al* 2006, Rennenberg *et al* 2009, Butterbach-Bahl *et al* 2013).

However, a series of arguments and recent observations question the old paradigm (figure 1(a)) of a predominance of organic N cycling in permafrost-affected soils with little importance of mineral N cycling.

(a) First, increasing evidence shows that emissions of the potent GHG N_2O are more important for permafrost-affected soils than previously thought (Voigt *et al* 2020). Since about a decade, large N_2O emissions have been reported from a wide range of permafrost-affected ecosystems (Repo *et al* 2009, Elberling *et al* 2010, Voigt *et al* 2020). As N_2O emissions are largely originating from various nitrification and denitrification pathways as well as chemical decomposition of nitrification intermediate products such as hydroxylamine (Butterbach-Bahl *et al* 2013), this observation points to a larger importance of mineral N cycling in permafrost-affected soils.

(b) Second, several studies showed high annual gross ammonification and nitrification at relatively low annual temperatures (e.g. in continental steppe of Inner Mongolia; Wu *et al* 2012), with freeze-thaw events being a hot moment for N mineralization and emissions of N gases (Wolf *et al* 2010, Wu *et al* 2012, Wagner-Riddle *et al*
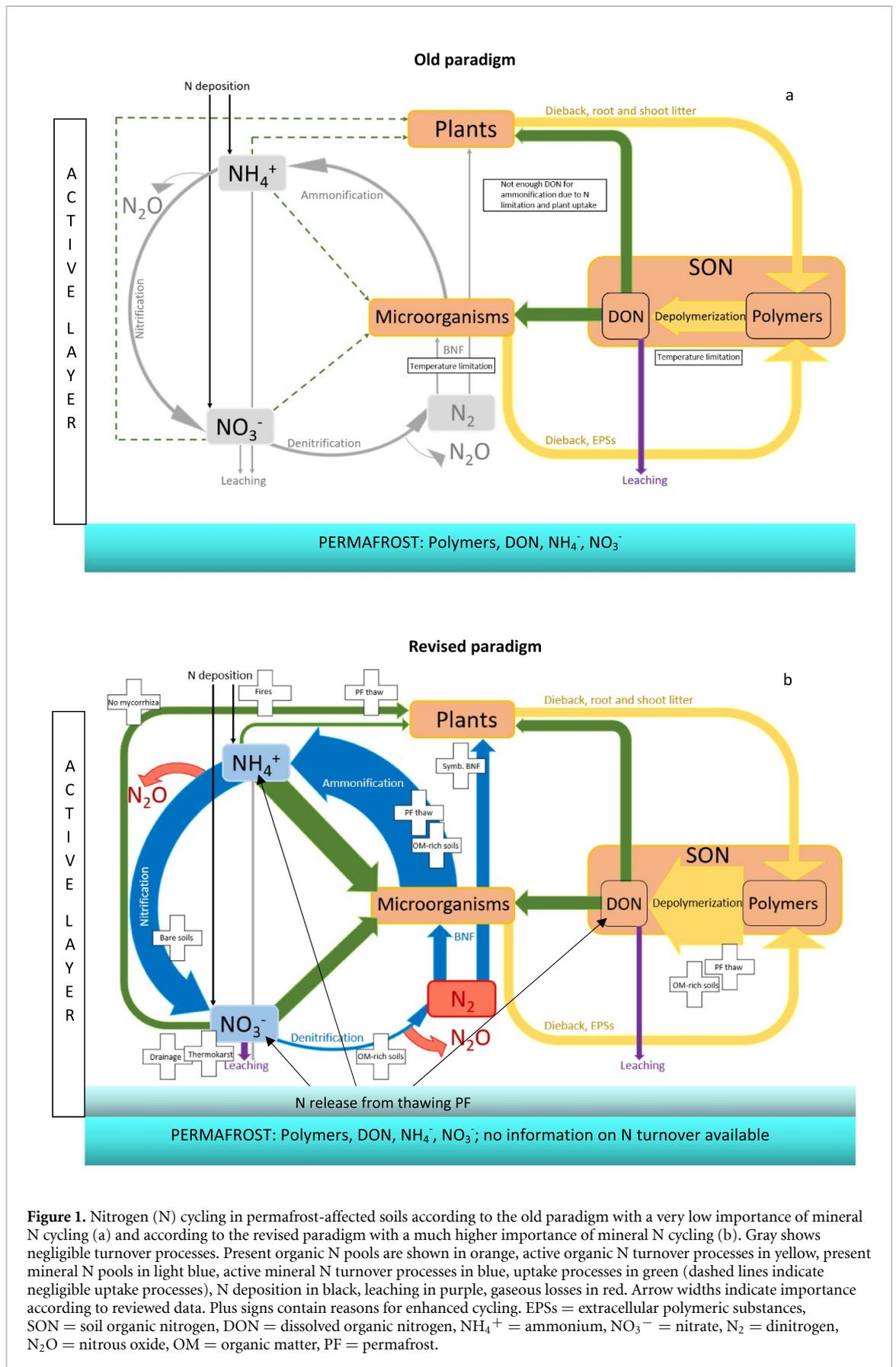
Figure 1. Nitrogen (N) cycling in permafrost-affected soils according to the old paradigm with a very low importance of mineral N cycling (a) and according to the revised paradigm with a much higher importance of mineral N cycling (b). Gray shows negligible turnover processes. Present organic N pools are shown in orange, active organic N turnover processes in yellow, present mineral N pools in light blue, active mineral N turnover processes in blue, uptake processes in green (dashed lines indicate negligible uptake processes), N deposition in black, leaching in purple, gaseous losses in red. Arrow widths indicate importance according to reviewed data. Plus signs contain reasons for enhanced cycling. EPSs = extracellular polymeric substances, SON = soil organic nitrogen, DON = dissolved organic nitrogen, NH_4^+ = ammonium, NO_3^- = nitrate, N_2 = dinitrogen, N_2O = nitrous oxide, OM = organic matter, PF = permafrost.

2017, Wu *et al* 2020). A study conducted in the German Alps even showed the occurrence of large gross ammonification and nitrification rates in frozen montane grassland soil, both based on measurements of gross N turnover and molecular analysis of functional N cycle genes

(Wang *et al* 2016). Consequently, the temperature conditions in permafrost-affected soils do not per se exclude significant mineral N production and turnover. Meanwhile, also studies on gross N turnover in permafrost-affected soils were published, but the information is scattered and has not yet been synthesized.

(c) Third, the paradigm that ammonification is largely insignificant in the C- and N-rich permafrost-affected soils contradicts our general understanding of ecosystem controls apart from permafrost-related studies, i.e. a positive correlation of soil organic carbon (SOC) and total nitrogen (TN) concentrations with gross ammonification (Booth *et al* 2005). Furthermore, Wild *et al* (2015) did not detect a decrease in microbial N limitation along a latitudinal transect ranging from arctic to temperate ecosystems. Instead, N limitation seems to decrease with soil depth (Meyer *et al* 2006, Wild *et al* 2015).

(d) Fourth, it is well known that biological N fixation (BNF) can significantly contribute to N availability in permafrost ecosystems (Henry and Svoboda 1986, Hobara *et al* 2006, Stewart *et al* 2013). Given the large BNF rates of cyanobacterial associations in permafrost-affected soils (0.1–25.8 kg N ha^{-1} yr^{-1}; table 2), this additional N input could lift N limitation for microbes, thereby facilitating gross ammonification (Stewart *et al* 2014).

(e) Fifth, atmospheric N deposition might still be generally low in the northern circumpolar permafrost region, but is expected to rise due to increased emissions from global and local sources (Lamarque *et al* 2005, Dentener *et al* 2006). Especially the increasing frequency of severe fires in the Arctic (Holloway *et al* 2020) could increase atmospheric N loads in this region at large scales, as was recently reported for tropical regions of Central Africa (Bauters *et al* 2018).

(f) Sixth, the Arctic is warming rapidly and climate change related disturbances are becoming more common (Biskaborn *et al* 2019, IPCC 2019). Ammonium/nitrate content is usually an order of magnitude higher in the permafrost than in the active layer (Keuper *et al* 2012, Beermann *et al* 2017, Fouché *et al* 2020). Warming of permafrost regions and subsequent permafrost thaw may promote N availability through C and N substrate release from formerly protected permafrost. Such increased N availability, together with increased CO_2 concentrations in the atmosphere, might increase vegetation cover in permafrost ecosystems and associated rhizodeposition of labile C and N compounds that again—via priming of soil organic matter (SOM) decomposition—could enhance mineral N cycling.

These points indicate that mineral N cycling in permafrost-affected soils has possibly been underestimated. Hence, here we attempt to assess and quantify the role mineral N cycling plays in permafrost-affected soils. In order to reflect on the validity of the classical permafrost N cycling paradigm, we synthesize existing data on gross and net N turnover rates in the plant-soil-microbe system, as well as N input and output processes. Overall, our objective is to characterize the significance of permafrost mineral N cycling and to identify research gaps that hamper understanding permafrost ecosystem N cycling under current and future climate.

2. Methods

We conducted a meta-analysis to synthesize current knowledge on gross and net N turnover rates in permafrost-affected soils. This was accompanied by literature reviews on soil organic N properties, N inputs by BNF and atmospheric N deposition, on the importance of mineral versus organic N in plant N nutrition, and on gaseous/hydrological N losses. Peer-reviewed papers referring to net and gross N turnover rates (net DON production/gross protein depolymerization, net/gross ammonification, net/gross nitrification) under field and laboratory conditions were collected from the Web of Science using specific search terms (for more details on literature survey, data extraction and assembly please see supplementary material 1 available online at stacks.iop.org/ERL/17/013004/mmedia). Additional studies were found from links to other publications and based on the expert knowledge of the authors. In total 16 studies were suitable for our analysis of gross N rates and 33 studies for our analysis of net N rates (see meta-analysis references, supplementary material 1). From that we extracted 81 datasets concerning gross rates and 163 datasets concerning net rates (often more than one turnover process was measured). The data were extracted from tables or graphs (using GetData Graph Digitizer 2013). In the following, we refer to net changes of DON and amino acids as net DON production. Soils were classified as organic or mineral as reported in the papers or based on C and N concentrations and C:N ratios (if possible). Further data on climate and soil properties were extracted (see supplementary material 1) to analyze potential controls of N transformation rates. Graphs and linear regressions were produced in Origin Version 2016 (OriginLab Corporation 2016). The regression lines do not account for standard errors as this might bias the data due to only few available standard errors. Relative importance of environmental factors controlling gross N turnover rates were assessed using stepwise

multiple linear regression analysis which was conducted in SPSS 8.0 (SPSS Inc 1998) and a tool for proportional marginal variance decomposition which was performed in R v3.2.6. (R Core Team 2019) using the R package *relaimpo* (see Grömping 2021). Statistical significance was given at 95% confidence interval. Gross rates of N turnover in permafrost-affected soils were compared with respective data of temperate/tropical systems by use of the gross N turnover datasets published by Booth *et al* (2005) and Elrys *et al* (2021a, 2021b). For this purpose, also data contained in graphs of Booth *et al* (2005) were digitized and data from Elrys *et al* were obtained via personal communication.

BNF rates reported for mosses/lichens/cyanobacteria and alders in permafrost systems were extracted from the Web of Science and based on scientific expertise of the authors. If expressed in other units, the rates were converted to kg N ha^{-1} yr^{-1} (or per season) since this is the common unit in the scientific field, notwithstanding that the observed areal coverage of N$_2$-fixing vegetation communities of mosses/lichens/cyanobacteria is often patchy and they vary at much smaller scales than one hectare.

All mentioned processes are driven by microbial activities. Despite much is known about drivers for microbiomes in temperate systems and factors determining their activities, it is unclear if the available data can be used to improve our understanding on N turnover in permafrost-affected soils, mainly as the keystone species for the different processes might differ, with different ecophysiologies compared to those microorganisms which we are already well aware off. This also relates to microbial network formation and interaction patterns, which are essential mainly for nitrification but to a smaller extent also for other processes. We used the small amount of literature available where phylogeny of functional groups was described and linked these data to known properties of the described taxa also from other cold environments.

3. How important is microbial mineral N production in permafrost-affected soils?

The perception that N cycling in permafrost-affected soils is largely based on organic N forms has been promoted by many studies quantifying soil dissolved organic and mineral N concentrations and/or net rates of N turnover. This is because dissolved N concentrations and net rates of N turnover are relatively simple to determine and therefore preferably analyzed in arctic environments compared to gross rates of N turnover, which require elaborate stable isotope studies. While gross rates reveal rates of single N turnover processes, net rates reflect the balance of production and consumption of an N compound, thereby integrating several gross processes.

3.1. A synthesis of published rates of gross and net N turnover in permafrost-affected soils

Meta-analysis of N turnover rates published for permafrost-affected soils (mg N kg sdw^{-1} d^{-1}; net rates N = 247 observations, gross rates N = 132 observations) in field or laboratory studies revealed strikingly different patterns in net and gross transformation rates. Net DON production rates (only available for six organic soils with mean of −9.4 and median of −2.9 mg N kg sdw^{-1} d^{-1}) were negative while mean and median net rates of ammonification, nitrification and N mineralization were either slightly negative or close to zero with no significant differences between organic and mineral soil horizons (figure 2(a)). The mean net DON production of all reviewed studies, including those that could not be categorized as organic or mineral, was −5.1 mg N kg sdw^{-1} d^{-1} (median: 0.02; N = 11) (figure S1). The mean net N mineralization was 0.8 mg N kg sdw^{-1} d^{-1} (median: 0.1; N = 127) (figure S1), which appears to confirm the absence of significant mineral N cycling in permafrost-affected soils. To compare, Liu *et al* (2017) summarized the average net mineralization over North and South America, Europe, Africa, Asia and Oceania across different ecosystems to be 2.4 ± 0.2 mg N kg sdw^{-1} d^{-1}. Shrub ecosystems showed the lowest net N mineralization rates (0.4 ± 0.1 mg N kg sdw^{-1} d^{-1}) and wetlands the highest net mineralization rates (6.1 ± 1.7 mg N kg sdw^{-1} d^{-1}). Under the traditional plant-nutrition-oriented view of net rates as a measure of the N 'left over' by microbes for plant N uptake, the low net mineralization in permafrost areas would confirm that plants need to rely on organic N sources. However, the negative net DON production rates also suggest high microbial immobilization of DON (figure 1(a)) and thus a very strong and successful microbial competition for DON against plants.

While the hardly detectable net rates tell that mineral N production in permafrost-affected soils might be generally negligible, the gross rates show that this is clearly a misconception (figure 2(b)). Mean gross protein depolymerization in permafrost-affected soils as obtained from published studies was 69.6 mg N kg sdw^{-1} d^{-1} (median: 8.7; N = 30), mean gross ammonification was 13.8 mg N kg sdw^{-1} d^{-1} (median: 3.1; N = 65) and mean gross nitrification 6.6 mg N kg sdw^{-1} d^{-1} (median: 1.9; N = 51) (figure S2), with organic soils showing considerably larger rates than mineral soils (figure 2(b)). Gross N turnover rates did not significantly differ between field and laboratory studies (figure S3).

Hence, our synthesis of N turnover data clearly shows significant gross ammonification and nitrification activity in permafrost-affected soils, which is not at all reflected by the negligible rates of net N turnover. Also for other N-limited systems, such mismatches of net and gross rates were highlighted. For instance, Wu *et al* (2012) showed that an annual
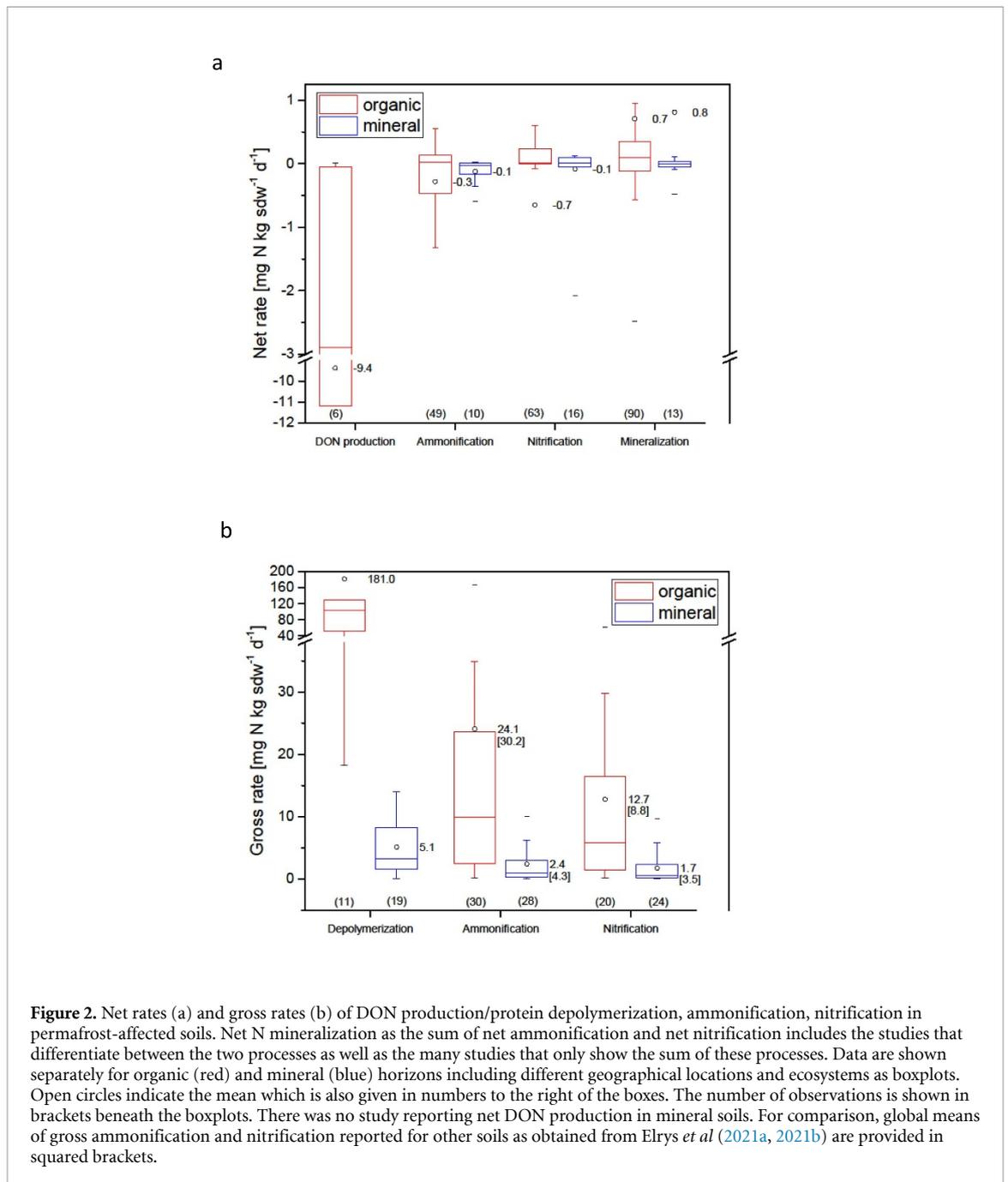
Figure 2. Net rates (a) and gross rates (b) of DON production/protein depolymerization, ammonification, nitrification in permafrost-affected soils. Net N mineralization as the sum of net ammonification and net nitrification includes the studies that differentiate between the two processes as well as the many studies that only show the sum of these processes. Data are shown separately for organic (red) and mineral (blue) horizons including different geographical locations and ecosystems as boxplots. Open circles indicate the mean which is also given in numbers to the right of the boxes. The number of observations is shown in brackets beneath the boxplots. There was no study reporting net DON production in mineral soils. For comparison, global means of gross ammonification and nitrification reported for other soils as obtained from Elrys *et al* (2021a, 2021b) are provided in squared brackets.

dataset of net rates of N turnover in continental semi-arid steppe soils of Inner Mongolia did neither reflect plant N availability nor the enormous magnitude and seasonal dynamics of gross inorganic N production rates. All of this is explained by the fact that net rates integrate all producing and consuming processes, e.g. of NH_4^+ or NO_3^- (usually in absence of plants using non-intact soil cores). Thus, net rates do not reflect rates and dynamics of mineralization-immobilization turnover and ignore major plant-soil-microbe interactions, which is not in line with a modern perception of profound plant impacts on N turnover via successful competition for N also in N-limited systems (Rennenberg *et al* 2009, and references therein). Based on our synthesis we conclude that net rates of

N turnover are of comparably little use to provide insight into N cycling in permafrost-affected soils.

Only Wild *et al* (2013, 2015, 2017, 2018) reported gross protein depolymerization for permafrost-affected soils, while for temperate and tropical systems, hardly any depolymerization data are available. The available data on gross protein depolymerization in permafrost-affected soils, derived from Greenland, Sweden and Siberia, indicate that gross protein depolymerization exceeded gross ammonification on average by approximately an order of magnitude in organic soils, i.e. only a minor fraction of the produced DON was further mineralized to NH_4^+, however, at still notable rates (figure 2(b)). To compare, in temperate soil beech

litter (in Austria) gross protein depolymerization rates (131–497 mg N kg sdw^{-1} d^{-1}) exceeded gross ammonification 8.6–34.8-fold and gross nitrification 8.5–32.7-fold (Wanek *et al* 2010). Also Jones and Kielland (2002) observed for Alaskan black spruce taiga that ammonification was slower than depolymerization. Such findings suggest that the bottleneck of N mineralization in permafrost areas is not the transformation of high-molecular-weight DON to low-molecular-weight DON, but the transformation of low-molecular-weight DON to NH_4^+. This means that microbes invest relatively much in the depolymerization of N-rich polymers (N mining) via extracellular enzymes in order to overcome N limitation. Generally, information on gross protein depolymerization comes with the caveats that only few data have been published and that the high published rates suggest a possibly questionable, very low mean residence time of depolymerized soil organic N (SON; the difference between TN and inorganic N) of only weeks to months. The underlying methodology of isotopic dilution of ^{15}N-labeled amino acids has been simplified recently (Noll *et al* 2019) so that more data might become available in near future to shed further light on the quantitative role of depolymerization in permafrost-affected soils. Based on the available data, we conclude that the reported very high gross protein depolymerization rates in permafrost-affected soils contradict two major N cycle paradigms, i.e. the assumptions that (a) depolymerization is the limiting 'bottleneck' of the N cycle and (b) that depolymerization in permafrost-affected soils is strongly limited by temperature (Schimel and Bennett 2004). These high rates challenge the perception of ubiquitous N limitation in northern soils (Wild *et al* 2015), but suggest a rapid turnover of at least part of polymeric organic matter. Nonetheless, the extent to which either the transformation of proteins to amino acids or of amino acids to NH_4^+ control N availability in permafrost-affected soils remains severely understudied.

Our meta-analysis data on gross N turnover rates in permafrost areas refer to the active layer of a permafrost ecosystem except for one study. Mao *et al* (2020) compared an active layer (0–10 cm) to a permafrost layer (215–265 cm) on the Tibetan Plateau with the permafrost layer being analyzed for gross N turnover shortly after thawing. They found that gross ammonification (0.5 ± 0.04 mg N kg sdw^{-1} d^{-1}) and nitrification rates (0.1 ± 0.00 mg N kg sdw^{-1} d^{-1}) were lower in the permafrost layer than in the active layer (3.5 ± 0.16 and 0.6 ± 0.03 mg N kg sdw^{-1} d^{-1}, respectively), but still detectable, indicating substantial mineralization activity immediately after permafrost thaw (supplementary material 2). However, to our knowledge no published study to date has successfully determined gross N turnover under frozen permafrost soil conditions.

Permafrost gross ammonification and nitrification rates from the meta-analysis were compared with other systems using the comprehensive synthesis of gross N turnover by Booth *et al* (2005) who presented a wide range of gross N turnover rates measured in temperate, tropical, semiarid and arctic/montane ecosystems (only ca. 4% in arctic/montane systems). Since the original data were not available, we digitized data from Booth *et al* (2005) and found mean gross ammonification from organic and mineral soils to be 13.5 mg N kg sdw^{-1} d^{-1} (median: 4.7; N = 158), while mean gross nitrification was 3.0 mg N kg sdw^{-1} d^{-1} (median: 1.2; N = 158). Thus, mean permafrost gross ammonification (13.8 mg N kg sdw^{-1} d^{-1}) turned out to be comparable to and mean permafrost gross nitrification (6.6 mg N kg sdw^{-1} d^{-1}) even tended to be higher than in non-permafrost ecosystems. More recently, Elrys *et al* (2021a, 2021b) provided a global synthesis of gross ammonification and nitrification across climatic zones, thereby distinguishing between organic and mineral soils. Interestingly, the very recent global mean values of gross rates provided by Elrys *et al* do not significantly differ from synthesized data of Booth *et al* (2005). Elrys *et al* (2021a) reported mean global ammonification of 30.2 and 4.3 mg N kg sdw^{-1} d^{-1} for organic and mineral soils, respectively, which is well comparable to the permafrost data of this study (24.1 and 2.4 mg N kg sdw^{-1} d^{-1}; figure 2(b)). Global gross nitrification was 8.8 and 3.5 mg N kg sdw^{-1} d^{-1} in organic and mineral soils, respectively (Elrys *et al* 2021b), which is also comparable to the rates obtained for permafrost soils in this study (12.7 and 1.7 mg N kg sdw^{-1} d^{-1}; figure 2(b)).

When comparing gross ammonification rates of permafrost-affected soils collected within this study with those of other ecosystems presented by Booth *et al* (2005), it becomes evident that gross ammonification rates in permafrost-affected soils show not only a comparable magnitude, but also almost the same dependency on SOC and TN concentrations compared to rates in other ecosystems (figure 3). Specifically, gross ammonification increases at the same rate depending on SOC concentrations in permafrost-affected soils as in soils of other ecosystems (temperate/tropical/montane) (figure 3(a)). The dependency of gross ammonification and TN concentrations for permafrost-affected soils even shows a slightly steeper slope compared to data for other ecosystems (figure 3(b)).

This comparison suffers from the problem that published gross N turnover rates almost exclusively origin from snapshot studies that are mostly limited to a single or few sampling dates in the growing season. This caveat however does not only apply for permafrost studies. Hitherto, only few studies provided annual gross N turnover rates based on monthly or even more frequent sampling over an entire year (Wang *et al* 2016, and references therein). Hence,

Figure 3. Comparison of gross ammonification rates and their dependency on SOC (a) and TN (b) concentrations for permafrost-affected soils (in pink) and other soils (synthesized by Booth *et al*, part of figure 1). Symbol shapes of Booth *et al*'s data (in black): triangle = woody, circle = grass, star = agricultural. The permafrost gross rate studies include laboratory incubations (59%). The mean gross ammonification rates from Booth *et al* and from this review do not differ significantly. Booth *et al* (2005), John Wiley & Sons.

also the Booth *et al* (2005) dataset largely contains snapshot measurements mainly obtained during the warm season, as is the case for permafrost studies. Gross N turnover in winter remains a research gap in permafrost-affected as well as in other soils. This is particularly problematic as recent work suggests that both frozen soil and freeze-thaw events can be hot moments of N turnover (Wang *et al* 2016, Wu *et al* 2020). High dissolved mineral N concentrations in permafrost (Elberling *et al* 2010, Keuper *et al* 2012, Beermann *et al* 2017, Salmon *et al* 2018, Fouché *et al* 2020) could thus indeed indicate significant N mineralization, but no experimental evidence is available to support this.

A mean gross ammonification rate of 13.8 mg N kg sdw^{-1} d^{-1} (figure S2) at a soil depth of 0.1 m and a bulk density of 0.4 g cm^{-3} would in 100 d translate into a seasonal ammonification estimate of 552 kg N ha^{-1}, thereby exceeding the annual N demand of most temperate forests severalfold (Rennenberg and Dannenmann 2015). This rough but very conservative estimate of the average growing season potential of gross ammonification in permafrost systems suggests that, according to published rates, gross ammonification indeed can allow for significant mineral N nutrition of plants.

While gross nitrification on average accounts for about half of ammonification in permafrost-affected soils (figure 4), it is notable that gross nitrification is extensive in the organic layers in several ecosystems, even exceeding gross ammonification by orders of

magnitude in tundra soils of Greenland and Siberia (figure 4; supplementary material 2). These results point at a significant contribution of heterotrophic nitrification to the formation of NO_3^-, i.e. via a direct oxidation of organic N compounds to NO_3^- (Chen *et al* 2015). Furthermore, the results indicate a surprisingly high importance of autotrophic nitrification as fate of produced NH_4^+ in permafrost-affected soils, which is similar to or even higher than in other soils (figure 4). Since under N limitation, NH_4^+ is rather immobilized by heterotrophic microbes than nitrified (Butterbach-Bahl and Dannenmann 2012), the high relative importance of nitrification also contradicts the paradigm of strong N limitation.

3.2. Controls of gross N turnover in permafrost-affected soils

To identify and characterize controls of N transformation rates in permafrost-affected soils, we compared the N turnover rates with reported environmental, soil and vegetation parameters (supplementary material 2). Unfortunately, we could not consider soil temperature as the data were widely not reported. A parameterization of gross N turnover in permafrost-affected soils under different temperatures is among the most pressing research needs.

Stepwise linear regression (using sub-datasets of overlapping data on gross rates and potential controls, see included variables in table 1) with log-transformed data showed that gross protein depolymerization expressed on soil dry weight
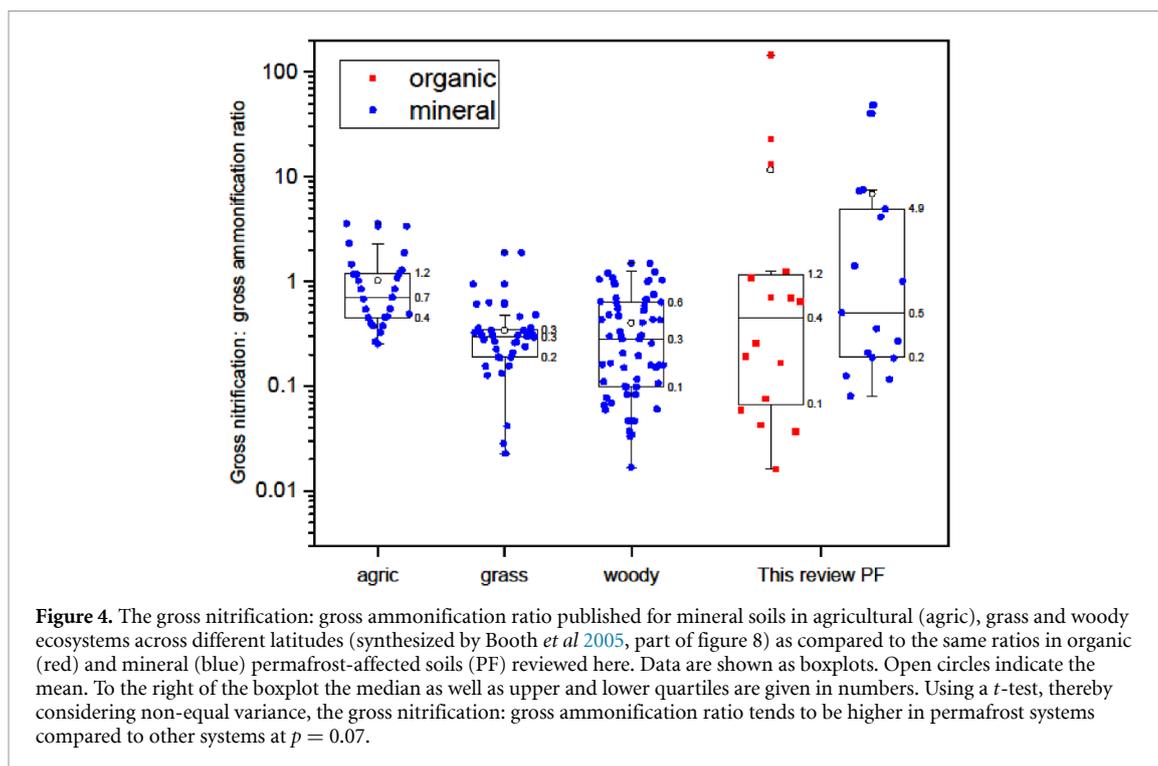
Figure 4. The gross nitrification: gross ammonification ratio published for mineral soils in agricultural (agric), grass and woody ecosystems across different latitudes (synthesized by Booth *et al* 2005, part of figure 8) as compared to the same ratios in organic (red) and mineral (blue) permafrost-affected soils (PF) reviewed here. Data are shown as boxplots. Open circles indicate the mean. To the right of the boxplot the median as well as upper and lower quartiles are given in numbers. Using a *t*-test, thereby considering non-equal variance, the gross nitrification: gross ammonification ratio tends to be higher in permafrost systems compared to other systems at $p = 0.07$.

Table 1. Stepwise linear regressions for gross protein depolymerization, ammonification and nitrification.

Gross N turnover	Included variables	Equation	*p*-value	R^2
Protein depolymerization	SOC, TN, C:N ratio	$1.1 \times LOG_{10} (SOC)$ $+0.1$	0.000 0.417	0.673
Ammonification	Gross protein depolymerization, SOC, TN, C:N ratio, DON	$0.9 \times LOG_{10}$ (gross protein depolymerization) $-0.7 \times LOG_{10}$ (TN) -1.2	0.000 0.018 0.000	0.608
Nitrification	Gross ammonification, SOC, TN, C:N ratio, DON, NH_4^+	$0.9 \times LOG_{10} (NH_4^+)$ -0.4	0.000 0.005	0.592

basis was mainly dependent on SOC concentration (table 1). Gross ammonification in turn was mainly controlled by gross protein depolymerization and TN concentration, with gross protein depolymerization explaining around 85% of gross ammonification (figure S4). Surprisingly, TN was correlated negatively with gross ammonification, suggesting that not TN, but only the active N fraction thereof (which can be easily decomposed) is important for gross ammonification. Gross nitrification was limited by NH_4^+ in the sub-dataset (table 1).

Analyzing the entire dataset of gross rates of N turnover and SOC/TN concentrations revealed that gross protein depolymerization, gross ammonification and gross nitrification rates were strongly positively correlated with SOC and TN concentrations (with the exception of gross nitrification versus SOC concentration) (figure 5). Our meta-analysis for permafrost-affected soils thus highlights SOC and TN concentrations as major controls of gross ammonification and nitrification which is in line

with the global synthesis by Booth *et al* (2005). Also Elrys *et al* (2021a) identified SOC and TN as positive controls of gross ammonification, besides a positive influence by increasing microbial biomass, increasing precipitation, decreasing bulk density and decreasing soil pH. This resembles results for potential net ammonification (Li *et al* 2019, 2020). Gross nitrification was influenced by TN, C:N ratio, microbial biomass, precipitation, temperature, soil pH and ecosystem types, which differed between heterotrophic and autotrophic nitrification (Elrys *et al* 2021b). These results show the importance of testing those factors again once there are more data for permafrost-affected soils.

Gross ammonification also was closely related to DON concentrations, indicating the relationship to its substrate (figure S5). Though suffering from limited data availability, the relationship between SOC concentrations and gross nitrification was best described by a polynomial function with mineral soils showing increasing nitrification with more SOC, but organic soils possibly showing decreasing

Figure 5. Gross protein depolymerization (a) and (b), gross ammonification (c) and (d) and gross nitrification (e) and (f) (mg N kg sdw^{-1} d^{-1}) versus SOC and TN concentration (%). Logarithmic scale (except for gross nitrification) and one common regression line for organic and mineral (pink). The dotted line shows a polynomial dependence which is considered to be speculative.
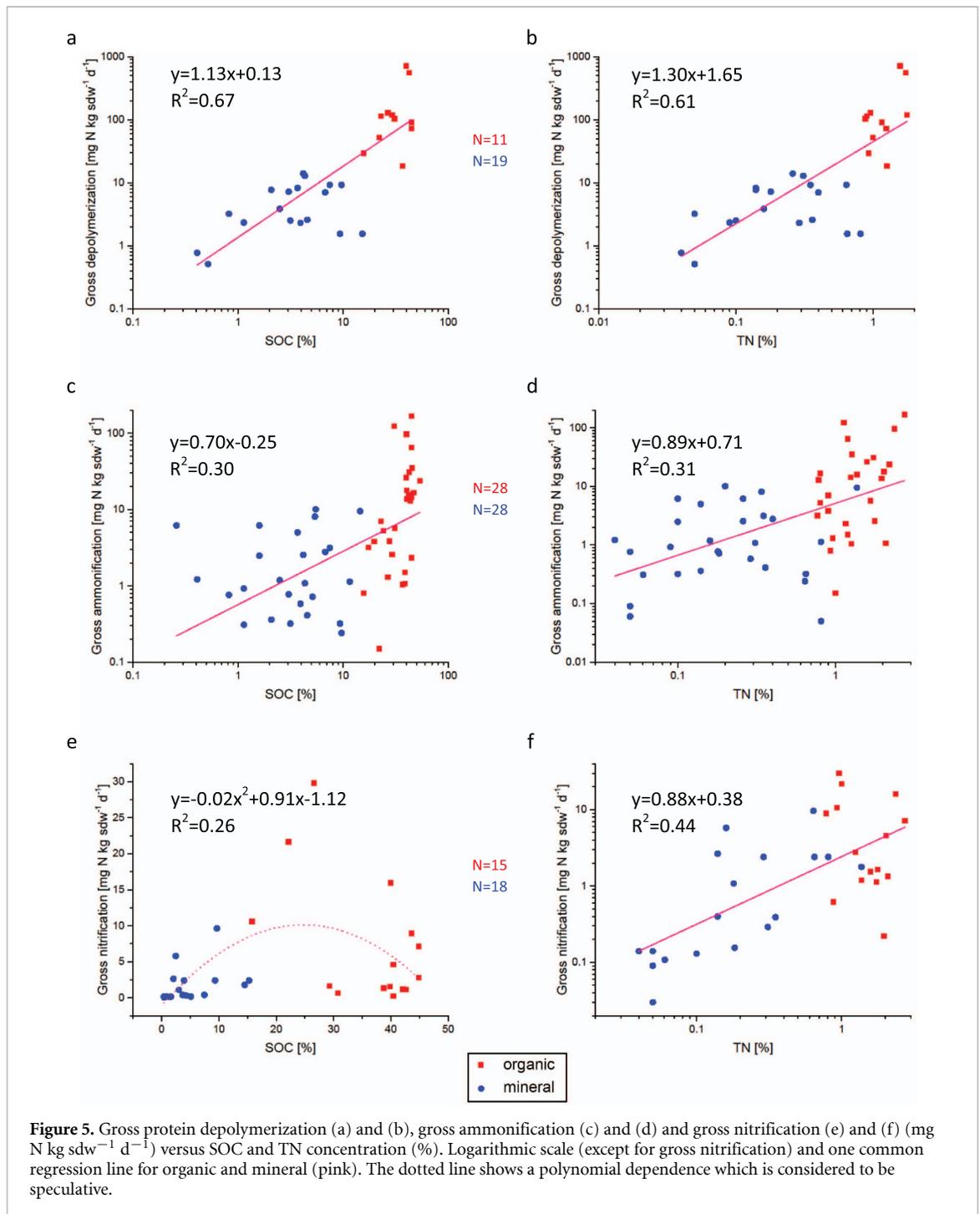
nitrification with more SOC (figure 5(e)). This might be explained by the autotrophic metabolism of many nitrifiers so that they do not rely on a C source. Furthermore, extremely high SOC concentrations in permafrost systems represent largely flooded peatland systems, where anaerobic processes dominate and nitrification might be inhibited by the lack of oxygen. The positive relationship of TN with gross nitrification (figure 5(f)) might reflect that with increasing N availability, NH_4^+ partitioning is increasingly directed in favor of autotrophic nitrification and at the expense of heterotrophic microbial NH_4^+

immobilization (Butterbach-Bahl and Dannenmann 2012).

The SOC:TN ratio (soil C:N ratio), has frequently been used as an indicator of N availability and to characterize N cycling, with high C:N ratios indicating low ammonification and nitrification but high microbial N immobilization and retention (Borken and Matzner 2004, Rennenberg *et al* 2009, Butterbach-Bahl and Dannenmann 2012). While the relationships mentioned above confirm a certain role of C:N stoichiometry in the regulation of ammonification and nitrification in permafrost-affected soils

as well, the influence of SOC and TN concentrations on gross N turnover prevails so that overall gross protein depolymerization and ammonification increased with increasing C:N ratios (figures S6 and S7) due to the correlation of SOC concentrations with C:N ratios. Also gross nitrification showed no negative relationship to C:N ratio (figure S8). Consequently, the C:N ratio was a poor indicator of gross N turnover in permafrost-affected soils. A prevalence of SOC over C:N ratios as dominant control of gross N turnover is however typical for across-ecosystem comparisons not only in permafrost ecosystems (Booth *et al* 2005). Notwithstanding this, at given C availability (especially dissolved organic carbon; DOC), changing N availability possibly can strongly influence gross N turnover. However, the use of DOC:DON ratios, i.e. a C:N index more related to bioavailability, did not indicate that for our dataset (figure S9). Also for net rates only an insignificant trend of organic soils towards larger net nitrification with lower C:N ratios was found (figure S10). In summary, based on the collected data it appears that C:N stoichiometry is—in contrast to expectations—not a decisive factor in explaining different N turnover across different permafrost-affected soils. In contrast, N mineralization in active layers seems to be much more related to absolute SOM content.

4. How available is SON in permafrost-affected soils for N mineralization?

With SOC and TN content being the main control, the tremendous amounts of SOM in permafrost-affected soils of the northern hemisphere (Tarnocai *et al* 2009, Hugelius *et al* 2014) facilitate high rates of gross N turnover upon thaw. High SOC and TN concentrations originate from long-term organic matter incorporation over millennia. The SOM accrual is thought to be further enhanced by reduced mineralization due to low temperatures and oxygen limitation, and in particular by physical protection of SOM from microbial attack in frozen soil (Harden *et al* 2012, Mueller *et al* 2015). Consequently, increased bioavailability of SOM due to reduced environmental constraints (e.g. higher temperatures, reduced permafrost) in active layers (Oechel *et al* 1995, 2000, Schuur *et al* 2008, 2015) supports and explains high gross N turnover rates as outlined in figure 2(b).

Focusing on the prominent mechanisms that stabilize SOM in permafrost-affected soils, (a) the saturation and stabilization due to freezing of SOM and (b) the translocation of plant-derived SOM into deeper soil horizons by cryoturbation, Harden *et al* (2012) reported modelled N stocks to 3 m soil depth ranging from 4.6 kg N m^{-2} in cryosols with low or absent cryoturbation to 7.5 kg N m^{-2} in C-rich permafrost-affected peat soils with strong cryoturbation. The

differences in N stocks due to cryoturbation clearly point to the high relevance of the depth distribution of organic N forms within the soil profile. A burial of rather fresh litter-derived SOM by cryoturbation leads to the accrual of SOM with higher C:N ratios at greater soil depth (Treat *et al* 2016a). The SOM in such cryoturbated pockets contains high amounts of rather undecomposed plant residues (particulate organic matter; POM) (Diochon *et al* 2013, Mueller *et al* 2015). This might be caused by a slowed-down protein depolymerization of SOM translocated to greater soil depths possibly due to low abundance of fungi, as reported by Wild *et al* (2013) for cryosols in Siberia.

Heterotrophic N turnover processes such as ammonification, immobilization and denitrification are depending on the availability of DOC as a labile substrate for microorganisms. This appears relevant for permafrost-affected soils as well—as indicated by a positive correlation between DOC and gross ammonification in our meta-analysis dataset (figure S11). The DOC that leaches from plant residues is rich in rather labile, easily bioavailable SOM compounds (rich in carbohydrates, low in aromatic/aliphatic C) (Surey *et al* 2020). Thus, the large storage of POM in the form of plant residues as well as of mineral-associated organic matter in permafrost-affected soils (Gentsch *et al* 2015, Mueller *et al* 2015) might explain the substantial gross mineral N turnover revealed by our synthetic analysis. The release of soluble and, thus, more bioavailable SOM and SON is also directly affected by physical factors like the frequency of freeze-thaw cycles that have been shown to directly lead to an increased release of DOC and thus increased microbial activity including N$_2$O losses (Cui *et al* 2016, Yang *et al* 2016, 2018).

Especially DON released from SOM depolymerization at greater permafrost soil depth will have considerable implications, as it is hardly reached by plant roots and thus rather prone to ammonification and losses via denitrification or leaching (Koven *et al* 2015). With receding permafrost, the release of N by the decomposition of SOM will on the one hand occur in surface soils that are rich in organic matter (i.e. in the active layer where microbial communities are present) due to higher temperatures, and on the other hand in deep-soil N-rich SOM due to increased decomposition (Salmon *et al* 2018). With ongoing permafrost collapse and the alteration of intact permafrost landforms into water-logged sites (e.g. from permafrost peatland to permafrost-free fen), releasing previously stored SOM (Patzner *et al* 2020), the newly available N from SOM decomposition will also become more plant-available due to shifting vegetation and increasing rooting depth (Finger *et al* 2016, Blume-Werry *et al* 2019, Hewitt *et al* 2019, Pedersen *et al* 2020). In a warmer future, fostered root growth and elevated CO$_2$ mixing ratios might

further promote rhizodeposition of both labile C and N compounds, which could through priming of native SOM decomposition lead to further release of soil C and N (Rousk *et al* 2016a, Blume-Werry *et al* 2019).

Moreover, the availability of phosphorus (P) is of ample importance for the fate of SOM and the release of N by microbial activity, as microorganisms have a constrained C:N:P stoichiometric demand (Mooshammer *et al* 2014). It was demonstrated that P availability, which differs greatly between arctic ecosystems (Giesler *et al* 2012, Wild *et al* 2013), appears to be a co-limiting factor for the microbial utilization of SOM in the Arctic. In a warming Arctic with greater thaw depths and increased plant growth, P availability might have a stronger effect than N on the storage and decomposition of SOC, while there will be a net shift of nutrients from SOM into the vegetation (Jiang *et al* 2016).

The C:N ratios in permafrost-affected soils are considerably lower with increased intermixing of mineral material and thus with increasing soil depth from the organic peaty topsoils to mineral subsoils (Kuhry and Vitt 1996, Mueller *et al* 2015), with very low C:N values in deeper mineral-rich permafrost layers like Yedoma deposits (Strauss *et al* 2015), reflecting the decomposition degree of the stored SOM and possible degradation of SOM prior to sedimentation (e.g. Yedoma). It is well known for soils of the temperate zone that a decrease in C:N ratios with progressing decomposition demonstrates the enrichment in microbially immobilized N (Lehmann and Kleber 2015, Kallenbach *et al* 2016, Kopittke *et al* 2018, 2020), and this can also be assumed for permafrost-affected soils with low C:N ratios. High C:N ratios are known to indicate lower decomposability of fresh SOM due to the nutrient demand of decomposers, while with progressing decomposition C:N ratios become lower due to the loss of C and the microbial retention of N (Schädel *et al* 2014). Our data synthesis supports the occurrence of high depolymerization and ammonification under conditions of high SOC and TN concentrations that go along with high C:N ratios. This might reflect the high need for microbial N mining in arctic organic topsoils with high C:N ratios (Lavoie *et al* 2011, Sistla *et al* 2012). For permafrost-affected soils with rather low C:N ratios of the Tibetan Plateau an increase in N availability was demonstrated to lead to a lowering of the microbial priming of SOM decomposition due to a decreased need for N mining (Chen *et al* 2018). While the C:N ratios of permafrost-affected soils have been known to be strongly positively correlated with the release of SOC at accelerated permafrost retreat (Schädel *et al* 2014, Kuhry *et al* 2020), our data suggest SOC concentration to be a major predictor of the vulnerability of permafrost SOM to N mineralization as well, possibly due to the close link of depolymerization and ammonification.

5. The microbiome of permafrost-affected soils and its role in mineral N cycling

The production and turnover of mineral N in permafrost-affected soils requires a microbial community which is capable to survive and even grow under the psychrophilic conditions of permafrost-affected soils. Microbes developed a number of unique properties during evolution, which helped them to tolerate cold temperatures. The lower limit of microbial activities is $-20\,°C$ (D'Amico *et al* 2006), although survival of most spores etc is also possible below that temperature. So even at soil temperatures below $-4\,°C$, which are typically occurring in soils affected by continuous permafrost, microbial activities are obvious. At such temperatures, microbes depend on small amounts of unfrozen water present in the particular environments and require physiological adaptations (D'Amico *et al* 2006, Mackelprang *et al* 2017). Microbial communities involved in mineral nutrient cycling are abundant in permafrost-affected soils both in the active (e.g. Yergeau *et al* 2010, Lamb *et al* 2011, Alves *et al* 2013) and permafrost layers (e.g. Hultman *et al* 2015). In the face of the diverse needs for adaptation of microbiota to survive in permafrost-affected soils, the high diversity of microbiota found in such environments (Pikuta *et al* 2005, Vishnivetskaya *et al* 2006, Nicholson *et al* 2013, Frank-Fahle *et al* 2014, Frey *et al* 2016, Monteux *et al* 2018, Ivanova *et al* 2020) is surprising but in line with our synthesis of significant gross rates of mineral N turnover. Qi *et al* (2017) analyzed samples from an altitude gradient along the Tibetan Plateau and showed that microbial functional diversity and the number of unique genes increased with elevation. However, it must be taken into account that possibly not all deoxyribonucleic acid (DNA) in the frozen soil has been derived from living microbiota, as DNA from dead microorganisms might be highly persistent in such environments and thus the molecular analysis might include also a history of microbes which have been present at the respective sites in the past together with ones being dormant or actually active (Burkert *et al* 2019).

Most of the genera obtained by cultivation by Vishnivetskaya *et al* (2006) were capable of denitrification as proven by whole genome sequences. This observation was confirmed by a microcosm study which analyzed the effects of cryoturbation in an artic peatland soil using molecular tools (Palmer *et al* 2012). Quantitative polymerase chain reaction (PCR) revealed a higher abundance of bacteria harboring the nitrate reductase *narG* in cryoturbated than in unturbated peat soil. Bacteria capable to perform nitrite reduction (based on the abundance of the *nirS* and *nirK* genes) were also increased in the crypoturbated settings. Interestingly, always the bacteria harboring the *nirS* gene dominated over those carrying

the *nirK* gene. The importance of *nirS*-type denitrifiers in permafrost-affected soils was confirmed in a number of studies from different natural permafrost-affected soils in the northern hemisphere and in alpine soils (Andert *et al* 2012, Palmer and Horn 2015, Chen *et al* 2019). Global warming may even increase the abundance of *nirS*-type denitrifiers, mainly in the rhizosphere of shrubs and other plant species (Song *et al* 2021). In the study of Palmer *et al* (2012), numbers of bacteria capable to transform N_2O into N_2 were significantly lower compared to nitrite reducers, which also explains the strong increase of N_2O emissions in the cryoturbated soils after NO_3^- addition. However it must be considered that in the mentioned study only those bacteria were assessed which belong to the clade 1 of *nosZ*, and not those which harbor the *nosZ* genes of clade 2, due to the selection of the primers for analysis (Yoon *et al* 2016). Calderoli *et al* (2018) demonstrated the importance of clade 2 of the *nosZ* gene for N_2O reduction in permafrost-affected soils. The authors analyzed sediments from Ushuaia Bay, a subantarctic environment, and found that the majority of *nosZ* genes identified belong to clade 2 and could be assigned to different bacterial lineages. The analysis of a fosmid metagenomic library from the same site showed that the genomic context of clade 2 variants of *nosZ* variants was variable, and was accompanied by distinct regulatory elements, suggesting the evolution of differential ecophysiological roles. In a recent study by Hetz and Horn (2021) this observation was confirmed and a strong co-occurrence between *Rhodanobacter* spp. and taxa of the Burkholdericaceae was found. The authors considered *Burkholderiaceae* which harbor *nosZ* genes of clade 2 as key acetate assimilators during complete denitrification in acidic cryoturbated peat of the arctic tundra mainly at pH levels <4. Taking these observations together, it is obvious that the potential for denitrification is well-presented in permafrost-affected soils and functional redundancy for the different groups is high, which strongly contributes to the resilience.

Also the presence of nitrifiers in permafrost-affected soils is well-documented not only by turnover data but also by abundance measurements of the ammonia- and nitrite-oxidizing microbiota. Based on a recent study from Sanders *et al* (2019) in which permafrost-affected soils from Siberia were studied, nitrifiers represent 0.6%–6.2% of the total microbial community, as shown by 16S ribosomal ribonucleic acid (rRNA) amplicon sequencing. These numbers are significantly higher compared to what has been described for soils from temperate or tropical regions (Mukhtar *et al* 2019). Based on the analysis of the ammonia monooxygenase gene (*amoA*), ammonia-oxidizing bacteria (AOB) were found in nearly all soil types, whereas ammonia-oxidizing archaea (AOA) were only detected in soils with low SOM (Sanders *et al* 2019). This finding contradicts

a number of other studies, where the importance of AOA was proven under certain settings. For example, a recent study shows that only a few AOA species closely related with *Candidatus* Nitrosocosmicus spp. are fueling nitrification in acidic permafrost peat soils across several arctic sites (Siljanen *et al* 2019). Laanbroek *et al* (2018) could demonstrate that AOA were significantly more abundant in Brown than in Histic Andosols, while the opposite was observed for AOB when freshly sampled Icelandic Andosols affected by permafrost were analyzed. However, only the numbers of AOB but not the numbers of AOA correlated significantly and positively with potential NH_3 oxidation activities. Also, Alves *et al* (2013) who investigated arctic soils demonstrated that AOA were the only ammonia oxidizers detected in five out of eleven soils and that they outnumbered AOB in four of the remaining six. Banerjee and Siciliano (2012) observed strong spatial heterogeneities of AOA and AOB which they could relate to differences in SOC and moisture.

These findings may explain much of the contrasting data published on the abundance pattern of both redundant functional groups of ammonia oxidizers. All studies were in line with the finding that despite high abundance of ammonia oxidizers in permafrost-affected soils, their diversity is low, indicating a need for a special adaptation of AOA and AOB to psychrophilic environments. This was also confirmed by Hayashi *et al* (2020) who sampled soils from Langhovde, East Antarctica, and identified only six and ten operational taxonomic units for AOB and AOA, respectively. AOB were dominated by Nitrosospira, which is in line with data from Sanders *et al* (2019); Nitrososphaera and Nitrosocosmicus were the two dominant clusters of AOA. Two recent benchmark studies gave a first insight into the genomes of major ammonia oxidizers which are adapted to permafrost environments. Alves *et al* (2019) described the new species *Ca.* Nitrosocosmicus arcticus, a novel thaumarchaeon which was enriched from arctic soils. Genomic analyses show that this organism harbors all genes involved in NH_3 oxidation and in C fixation via the 3-hydroxypropionate/4-hydroxybutyrate cycle, characteristic of all AOA, as well as the capability for urea utilization and potentially also for heterotrophic metabolism. Interestingly, the authors observed faster growth rates (based on marker gene counts) at lower temperatures (4 °C–8 °C) but without detectable NO_2^- production. Sanders *et al* (2019) were able to enrich Nitrosospira-like AOB which made up to 50% of the diversity observed in their studies of permafrost-affected soils, and could confirm growth at lower temperatures.

Nitrifiers compete for NH_4^+ with plants in permafrost-affected soils. Indeed it has been shown that in vegetated permafrost peat activity of nitrifiers is limited by the competition for N with

vegetation, while the bare peat surfaces without vegetation have high nitrification activity (Repo *et al* 2009, Marushchak *et al* 2011, Voigt *et al* 2017a). But there is also strong competition for NH_4^+ with anammox bacteria, which utilize NH_3 under anoxic conditions. Zhao *et al* (2018) investigated the diversity, community composition, and abundance of anammox bacteria along an altitudinal gradient on the Qinghai-Tibet Plateau. A molecular detection revealed the presence of anammox bacteria mainly in samples which were more affected by freezing among the two types of soil samples. Results of high-throughput sequencing targeting the hydrazine synthesis β-subunit *(hzsB)* gene revealed the presence of three known anammox genera (*Ca.* Brocadia, *Ca.* Jettenia, and *Ca.* Kuenenia).

Generally, the high abundance of ammonia oxidizers in permafrost-affected soils indicates a relatively high importance of nitrification, thereby confirming findings of our meta-analysis on gross nitrification rates. While molecular analysis of ammonification is largely missing, the high abundance of ammonia oxidizers in permafrost-affected soils would be very surprising if there was no significant NH_3 production. Besides ammonification, an efficient recycling of NO_3^- via dissimilatory nitrate reduction to ammonium (DNRA) might also fuel nitrifiers. However, no clear evidence for a significant abundance and activity of DNRA in permafrost-affected soils exists so far. Most studies which described the mineral N cycle did not consider the *nrfA* gene, a marker for DNRA. Another possibility for nitrifiers is the alternative use of urea. Many ammonia oxidizers can utilize urea for nitrification because they possess the enzyme urease that hydrolyses urea to NH_3 and CO_2 (Pommerening-Röser and Koops 2005). The use of urea might be supported by 'reciprocal feeding' where urease-positive nitrite-oxidizing bacteria can provide urease for urease-negative AOB (Daims *et al* 2016). In this respect, also the role of comammox bacteria in permafrost-affected soils which are capable of the complete transformation of NH_3 into NO_3^- needs to be clarified. Of course, also the fixation of N_2 needs to be considered as a possible path for NH_3 formation (see section 6).

Ammonia oxidization in active layers is responding to higher substrate availability, moisture and temperature (Alves *et al* 2013, Osborne *et al* 2016, Daebeler *et al* 2017). There are results showing that warming had a minor effect on microbial communities involved in nutrient cycling including N_2O production processes (Lamb *et al* 2011). However, according to other studies warming had induced changes in the abundance of genes (Mackelprang *et al* 2011, Penton *et al* 2016) and transcripts (Hultman *et al* 2015) and increased N_2O emissions from permafrost peatland (Voigt *et al* 2017b). According to a recent meta-analysis of 93 field warming studies, warming increased N mineralization, N_2O emissions

and DON, but did not affect the abundance of functional genes relevant for N cycling (Salazar *et al* 2020).

6. The role of BNF in permafrost-affected soils

Being an important N input source into soils, BNF is thought to play an important role by facilitating N limitation in the remote and pristine permafrost region (Chapin and Bledsoe 1992, Vitousek *et al* 2002, Hobara *et al* 2006, Lindo *et al* 2013, Stewart *et al* 2013). A major group of prokaryotes performing BNF are cyanobacteria. These phototrophic prokaryotes can be associated with certain plants like bryophytes or grasses as facultative epiphytes (Solheim *et al* 1996) or endophytes (Turetsky 2003), they can be an obligatory constituent of a lichen (cyanolichens) as symbionts, or free-living in water or topsoils. Another N_2-fixing group important in permafrost areas are actinorhizal root symbionts of trees in subarctic ecosystems close to the tree line or in permafrost-rich boreal landscapes.

How N_2 fixation influences nutrient availability for microbes and plants is not completely understood (Belnap 2001, Johnson *et al* 2005, Knowles *et al* 2006, Lagerström *et al* 2007, Stewart *et al* 2014), especially not for permafrost areas. Generally, N availability can be increased (Zielke *et al* 2005, Stewart *et al* 2011a, 2011b, 2013, Letendre *et al* 2019), but the question is if, when and how exactly BNF triggers ammonification and subsequent nitrification and denitrification with associated gaseous N losses. A prerequisite for BNF-induced N_2O production in an ecosystem seem to be low immobilization rates of microbes and plants, as otherwise the fixed N is immediately assimilated (Diáková *et al* 2016, Voigt *et al* 2020). For example, in arctic environments with high N limitation but N-saturated microorganisms and/or low abundance of plants, like in polar deserts, BNF can fuel N cycling from ammonification to N_2O emissions (Stewart *et al* 2013, 2014). Another possibility is that plants that are associated with N_2 fixers trigger N cycling and N_2O emissions themselves (e.g. potentially alders; see section 6.2).

However, since BNF and low N availability are strongly correlated due to down-regulation processes (Vitousek *et al* 2002), in many ecosystems N inputs via BNF are often readily taken up and immobilized by microbes and/or plants, preventing N_2O emissions. This is why one can find ecosystems with BNF that lack N_2O emissions (Diáková *et al* 2016). Vice versa, there are ecosystems with high N turnover rates and N_2O emissions, but without N_2 fixation, e.g. barren peat surfaces (Diáková *et al* 2016). N_2O emissions from permafrost ecosystems are thus not strictly dependent on BNF, but can be caused solely by internal cycling processes as well. When, however, the microbes or plants that immobilized the fixed N_2

die off, the previously immobilized N can become available in the soil in the form of organic N. This fits observations that a big share of dissolved N that N_2 fixers release is organic (Johnson *et al* 2005, Stewart *et al* 2014). Higher inputs of organic N due to BNF could, after being depolymerized, finally stimulate the production of NH_4^+ in the long term.

The persisting lack of knowledge on BNF rates is exacerbated by a prevailing use of acetylene reduction assays (ARAs) with controversial conversion factors, suitable for comparative studies, but resulting in doubtful absolute rate numbers. Publications using more reliable, direct $^{15}N_2$ fixation methods in permafrost ecosystems are scarce (e.g. Vile *et al* 2014, Rousk *et al* 2016b, 2017).

6.1. N fixation from bryophytes, lichens and free-living cyanobacteria

Taking the whole terrestrial (sub-)arctic into account, cyanobacteria are the primary N_2-fixing organisms (Henry and Svoboda 1986, Solheim *et al* 1996, Hobara *et al* 2006). Lichens do not account for as much BNF as bryophytes due to their lower mass, but their BNF rates per mass are often higher (Schell and Alexander 1973, Crittenden and Kershaw 1978, Gunther 1989, Hobara *et al* 2006), e.g. accounting for 24.9 kg N ha^{-1} yr^{-1} in a low arctic tundra (Stewart *et al* 2011a). As can be seen in table 2, reported BNF rates are higher than commonly thought. In subarctic regions, N_2 fixation rates by moss-associated cyanobacteria were found to range from 0.3 to 5 kg N ha^{-1} yr^{-1} (Rousk *et al* 2015, 2017). Values are even higher for frost-heaved sites: in subarctic Northern Sweden N_2 fixation accounted for an input of 8.8–11 kg N ha^{-1} season^{-1}, so that the fixed N exceeded annual plant uptake (Sorensen *et al* 2006). In peat of bogs in Alberta, Canada, the mean BNF rate was as much as 25.8 ± 2.4 kg N ha^{-1} yr^{-1} (Vile *et al* 2014). In high arctic ecosystems BNF rates are lower, but still account for 65%–90% of the total ecosystem N input (Henry and Svoboda 1986, Hobara *et al* 2006).

There are also N_2-fixing prokaryotes that are free-living in water/soil and form colonies. They often belong to particularly drought-resistant biological soil crusts (BSCs) together with algae, mosses, liverworts, fungi and lichens (Stewart *et al* 2014). Besides decomposition, direct N leakage from crust organisms is substantial (Evans and Lange 2001). For example, 1%–2% of TN in BSCs could be found extracellularly as NH_4–N in a semiarid desert in Arizona (Mayland *et al* 1966), but comparable data for arctic regions are missing. While bryophytes and lichens are assumed to release fixed N rather slowly due to decomposition or induced by disturbance (Rousk *et al* 2016b), free-living cyanobacteria release fixed N into the soil N pool within days to weeks including diffusion (Rousk *et al* 2016b) and BSCs do so even faster (Belnap 2001, Rousk *et al* 2016b).

Thus, they provide a constant N input into the soil. Although BNF rates by free-living cyanobacteria are generally low, NO and N_2O losses from steppe can be replaced by the fixed N_2 (Holst *et al* 2009). It is known that BNF still takes place at low temperatures (Dickson 2000, Arndal *et al* 2009), e.g. mats of *Nostoc* and *Calothrix* perfom BNF in soils at −4 °C, because photosynthesis is not stopped and cells are not entirely frozen (Davey 1983). A big portion of fixed N is oxidized within the BSCs (Johnson *et al* 2005), which might lead to gaseous N emissions under anoxic circumstances.

Fixation rates of 20–25 kg N ha^{-1} yr^{-1} argue for a release of N limitation over years and might contribute to the occurrence of mineral N turnover in active layers, while low BNF rates of less than 1 kg N ha^{-1} yr^{-1} might not change N availability of the ecosystem significantly. Our literature research led to a hypothetical mean BNF rate of 6.0 kg N ha^{-1} yr^{-1} by the different cyanobacterial associations (table 2; overall mean). There is a caveat concerning the reported BNF rates arising from the conversion of monthly or growing season rates to annual rates (ignoring times with lower BNF rates than during measurements). Thus, some rates in table 2 potentially overestimate actual rates.

A warming climate is expected to exert controversial effects on BNF rates. For instance, warming-induced increased shrub abundance in conjunction with increased N availability and turnover could diminish BNF rates (Zackrisson *et al* 2004, DeLuca *et al* 2007). On the other hand, BNF rates are likely to increase under conditions of increased temperature and soil moisture (Rousk *et al* 2018). Thus, there is a need for tracing the fate of fixed atmospheric N_2 into intact plant-soil-microbe systems as well as the use of molecular tools rather than ARAs (a) across various arctic ecosystems and (b) in controlled experiments including differences in moisture and temperature to finally reveal correlations between BNF rates and the N turnover including GHG emissions (Stewart *et al* 2013).

6.2. Symbiotic N fixation by trees (*Alnus-Frankia* association)

Evergreen shrubs, dwarf shrubs and grasses (including sedges) are the most common plant functional types in arctic wetlands, whereas the abundance of tree species is much lower (Bridgham *et al* 1996). Because ecosystems without trees are prevailing, overall N fixation in the arctic is dominated by bryophytes, lichens and soil crusts as outlined in section 6.1. However, due to their size any trees capable of BNF represent a very significant N input pathway with great potential to cause mineral N turnover and open the N cycle. Deciduous actinorhizal *Alnus* spp. constitute the only N_2-fixing tree species in arctic environments, often found close to the southern border of permafrost areas (Hibbs and Cromack 1990,

Table 2. BNF rates of associations with cyanobacteria or rhizobia and of the *Alnus–Frankia* association (mosses marked in green, lichens in yellow, free-living cyanobacteria in orange, BSCs in brown, legumes in dark green, alders in dark orange) as measured at different locations in different ecosystems of permafrost areas. Rates are given in kg N ha^{-1} yr^{-1} (or per season if mentioned). Sources and respective methods are shown.

Topic	Associations with N$_2$ fixers	BNF rate (kg N ha^{-1} yr^{-1})	Location	Ecosystem	Source	Method
6.1 N fixation from bryophytes, lichens and free-living cyanobacteria	Peat moss (*Sphagnum fuscum*) with cyanobacteria and methanotrophs	2.6	Near Lake Torneträsk, Abisko, Sweden (subarctic)	Wet heath, dry heath, polygon, forest, mire	Rousk *et al* (2015)	ARA, *in situ*
	Feather moss (*Hylocomium splendens*) with cyanobacteria	0.3	Near Lake Torneträsk, Abisko, Sweden (subarctic)	Wet heath, dry heath, polygon, forest, mire	Rousk *et al* (2015)	ARA, *in situ*
	Peat moss (*S. fuscum*)	1.0 ± 0.1	Near Abisko Scientific Research Station, Sweden (subarctic)	Bog	Rousk *et al* (2016b)	^{15}N$_2$ fixation, *in situ*
	Mosses with diazotrophs	5.0	Zackenberg, Greenland (high arctic)	Dry heath (dense moss surface)	Rousk *et al* (2017)	^{15}N$_2$ fixation, *in situ*
	Cotton grass and mosses with diazotrophs	1.2	Zackenberg, Greenland (high arctic)	Dry heath (water-logged fen)	Rousk *et al* (2017)	^{15}N$_2$ fixation, *in situ*
	Mosses with diazotrophs	1.2	Near Abisko Scientific Research Station, Sweden (subarctic)	Wet heath	Sorensen and Michelsen (2011)	ARA (conversion factor from earlier study), *in situ*
	Peat moss (*Sphagnum* spp.)	20.5	Daring Lake, Northwest Territories (low arctic)	Xerophytic herb tundra, heath-lichen/ heath-mat tundra, birch hummock, wet sedge meadow	Stewart *et al* (2011a)	ARA (calibrated by using ^{15}N$_2$ gas), *in situ* + modelling for upscaling
	Peat mosses (*S. fuscum, Sisyrinchium angustifolium, Sphagnum capillifolium*) with cyanobacteria and methanotrophs	25.8 ± 2.4	Alberta Bogs, Canada	Peat	Vile *et al* (2014)	ARA (calibrated by using ^{15}N$_2$ gas), *in situ*
	Cyanolichens	0.1	Brooks Lake, Alaska, USA	Tundra + boreal forest (Brooka Lake drainage)	Gunther (1989)	ARA, laboratory
	Cyanolichen (*Peltigera aphtosa*)	0.9	Near Lake Torneträsk, Abisko, Sweden (subarctic)	Wet heath, dry heath, polygon, forest, mire	Rousk *et al* (2015)	ARA, *in situ*

(Continued.)

Table 2. (Continued.)

Topic	Associations with N$_2$ fixers	BNF rate (kg N ha^{-1} yr^{-1})	Location	Ecosystem	Source	Method
	Cyanolichen (*P. aphtosa*)	3.5 ± 0.4	Near Abisko Scientific Research Station, Sweden (subarctic)	Heath	Rousk *et al* (2016b)	^{15}N$_2$ fixation, *in situ*
	Cyanolichen (*Stereocaulon paschale*)	24.9	Daring Lake, Northwest Territories (low arctic)	Xerophytic herb tundra, heath-lichen/heath-mat tundra, birch hummock, wet sedge meadow	Stewart *et al* (2011a)	ARA (calibrated by using ^{15}N$_2$ gas), *in situ* + modelling for upscaling
	Diazotrophs (including few cyanolichens *P. aphtosa*)	1.1	Imnavait Watersehd (Imnavait Creek), Alaska, USA (arctic)	Meadows	Hobara *et al* (2006)	ARA (calibrated by using ^{15}N$_2$ gas), laboratory + modelling for upscaling
	Thick moss mats, scattered lichens (mostly *P. aphtosa* and *Peltigera venosa*) with diazotrophs	8.8 per season	Shore of Lake Torneträsk, Abisko, Sweden (subarctic)	Frost-heaved moss-covered surfaces	Sorensen *et al* (2006)	ARA (calibrated by using ^{15}N$_2$ gas), *in situ* + laboratory
	Scattered mosses, liverworts, lichens (e.g. *P. aphtosa*) with diazotrophs	2.5 per season	Shore of Lake Torneträsk, Abisko, Sweden (subarctic)	Stable heath vegetation	Sorensen *et al* (2006)	ARA (calibrated by using ^{15}N$_2$ gas), *in situ* + laboratory
	Cyanobacteria (mostly Nostocales and Stigonematales)	24.5	Lais River + Vindel River + Pite River, northern Sweden (subarctic)	Alluvial meadow	DeLuca *et al* (2013)	ARA (calibrated by using ^{15}N$_2$ gas), *in situ*
	Cyanobacterial algae (especially *Nostoc* spp.)	0.8	Alexandra Fiord, Ellesmere Island, Canada (high arctic)	Sedge meadows	Henry and Svoboda (1986)	ARA, *in situ*
	Cyanobacterial algae (especially *Nostoc* spp.)	0.9	Sverdrup Pass, Ellesmere Island, Canada (high arctic)	Wet to mesic sedge-moss meadows	Henry and Svoboda (1986)	ARA, *in situ*
	Organic crust (free-living cyanobacteria)	0.9	Near Lake Torneträsk, Abisko, Sweden (subarctic)	Wet heath, dry heath, polygon, forest, mire	Rousk *et al* (2015)	ARA, *in situ*
	Cyanobacterial crust	0.6 ± 0.1	Near Abisko Scientific Research Station, Sweden (subarctic)	Polygon-patterned ground with open soil	Rousk *et al* (2016b)	^{15}N$_2$ fixation, *in situ*

(Continued.)

Table 2. (Continued.)

Topic	Associations with N_2 fixers	BNF rate ($kg\ N\ ha^{-1}\ yr^{-1}$)	Location	Ecosystem	Source	Method
	Organic crust (free-living cyanobacteria)	1.2	Zackenberg, Greenland (high arctic)	Dry heath	Rousk *et al* (2017)	$^{15}N_2$ fixation, *in situ*
	Organic crust (free-living cyanobacteria)	11.0 per season	Shore of Lake Torneträsk, Abisko, Sweden (subarctic)	Frost-heaved sorted circles	Sorensen *et al* (2006)	ARA (calibrated by using $^{15}N_2$ gas), *in situ* + laboratory
	Hollow BSC	3.3	Daring Lake, Northwest Territories (low arctic)	Xerophytic herb tundra, heath-lichen/heath-mat tundra, birch hummock, wet sedge meadow	Stewart *et al* (2011a)	ARA (calibrated by using $^{15}N_2$ gas), *in situ* + modelling for upscaling
	Hummock BSC	7.1	Daring Lake, Northwest Territories (low arctic)	Xerophytic herb tundra, heath-lichen/heath-mat tundra, birch hummock, wet sedge meadow	Stewart *et al* (2011a)	ARA (calibrated by using $^{15}N_2$ gas), *in situ* + modelling for upscaling
	Legume (*Astragalus alpinus*) with rhizobia	1.3	Near Lake Torneträsk, Abisko, Sweden (subarctic)	Wet heath, dry heath, polygon, forest, mire	Rousk *et al* (2015)	ARA, *in situ*
	Legume (*A. alpinus*) with rhizobia	4.2 ± 1.0	Near Abisko Scientific Research Station, Sweden (subarctic)	Transition zone between patterned ground and mixed dwarf shrub heath	Rousk *et al* (2016b)	$^{15}N_2$ fixation, *in situ*
	Overall mean	**6.0**				
	Overall median	**1.9**				

(Continued.)

Table 2. (Continued.)

Topic	Associations with N$_2$ fixers	BNF rate (kg N ha^{-1} yr^{-1})	Location	Ecosystem	Source	Method
6.2 Symbiotic N fixation by trees	*Alnus incana* spp. *tenuifolia* with *Frankia* bacteria	28–59	Tanana River, 35 km SW of Fairbanks, Alaska, USA (subarctic)	Taiga (floodplain)	Uliassi and Ruess (2002)	ARA incubation of nodules, *in situ*
	A. incana spp. *tenuifolia* with *Frankia* bacteria	140 ± 41	Tanana River, 35 km SW of Fairbanks, Alaska, USA (subarctic)	Taiga (floodplain), fertilized with phosphorous	Uliassi and Ruess (2002)	ARA incubation of nodules, *in situ*
	Alnus viridis spp. *fruticosa* with *Frankia* bacteria (late succession)	6.6 ± 1.2	Bonanza Creek Experimental Forest, 35 km SW of Fairbanks, Alaska, USA (subarctic)	Taiga (upland), post-fire succession	Mitchell and Ruess (2009)	ARA incubation of nodules (conversion factor from earlier study), *in situ*
	A. incana spp. *tenuifolia* with *Frankia* bacteria	22–107	S-N transect from Kenai Peninsula to Fairbanks (Quartz Creek, Eagle River, Tanana River), Alaska, USA (subarctic)	Taiga	Ruess *et al* (2009)	^{15}N$_2$ incubation of nodules, *in situ*
	A. incana spp. *tenuifolia* with *Frankia* bacteria	26–38	Bonanza Creek Experimental Forest, 35 km SW of Fairbanks, Alaska, USA (subarctic)	Taiga (floodplain)	Ruess *et al* (2013)	^{15}N$_2$ incubation of nodules, *in situ*
	A. viridis spp. *fruticosa* with *Frankia* bacteria	19.5 ± 6.8	Kougarok Hillslope, 103 km from Nome, Seward Peninsula, Alaska, USA (subarctic)	Tundra (alder shrubland community)	Salmon *et al* (2019)	^{15}N$_2$ incubation of nodules, *in situ*
	A. viridis spp. *fruticosa* with *Frankia* bacteria	5.3 ± 1.9	Kougarok Hillslope, 103 km from Nome, Seward Peninsula, Alaska, USA (subarctic)	Tundra (alder savanna community)	Salmon *et al* (2019)	^{15}N$_2$ incubation of nodules, *in situ*
	A. viridis spp. *fruticosa* with *Frankia* bacteria	33 ± 31	35 km NW and 40 km NE of Fairbanks, Alaska, USA (subarctic)	Taiga, post-fire succession (alder stand already before fire)	Houseman *et al* (2020)	^{15}N$_2$ incubation of nodules, *in situ*
	A. viridis spp. *fruticosa* with *Frankia* bacteria	91 ± 30	35 km NW and 40 km NE of Fairbanks, Alaska, USA (subarctic)	Taiga, post-fire succession (black spruce forest before fire)	Houseman *et al* (2020)	^{15}N$_2$ incubation of nodules, *in situ*

Pawlowski and Newton 2008). As pioneer species, alders generally increase soil fertility in the long term until successional species take over. BNF by *Alnus* spp. is mediated by its filamentous bacterial symbiont *Frankia* (Actinobacteria) at a range similar to *Rhizobium*-legume symbioses (Hibbs and Cromack 1990, Huss-Danell 1990, 1997, Dawson 2008). BNF of Siberian alder (*Alnus hirsuta* Turcz. var. *sibirica*) and other arctic alder species appears at budbreak, peaks at midsummer after full leaf expansion and disappears after all leaves have been shed, thereby following the seasonal change in nitrogenase activity of the nodules (Huss-Danell 1990, Tsutsumi *et al* 1993, Tobita *et al* 2013).

Temperate alder forests fix more than 150 kg N ha^{-1} yr^{-1} (Uri *et al* 2011). The annual amount of BNF of a Siberian alder forest stand in Japan was estimated at comparably low 56.4 kg N ha^{-1}, contributing 66.4% to the annual amount of N in leaf litter (Tobita *et al* 2013). Concerning permafrost areas data are very scarce and hitherto restricted to Alaska (table 2). In tundra, rates are comparably low: annual N_2 fixation was 5.3 ± 1.9 kg N ha^{-1} yr^{-1} by alder savannas and 19.5 ± 6.8 kg N ha^{-1} yr^{-1} by alder shrublands, with the latter still causing elevated N levels in adjacent plants and soils (Salmon *et al* 2019). Nitrogen input by late-succession *A. viridis* in upland of interior Alaska was 6.6 ± 1.2 kg N ha^{-1} yr^{-1} (Mitchell and Ruess 2009). However, *Alnus* spp. in floodplains can show much higher BNF rates of 22–107 kg N ha^{-1} y^{-1} (Ruess *et al* 2009). At a floodplain of the Tanana River, N_2 fixation in alder stands was 59 ± 11 kg N ha^{-1} yr^{-1} or 28 kg N ha^{-1} yr^{-1}, depending on the ARA conversion factor (Uliassi and Ruess 2002). Alder stands fixed even more N when fertilized with phosphorus (140 ± 41 kg N ha^{-1} yr^{-1}) (Uliassi and Ruess 2002). For boreal Alaska, it was estimated that after a fire with moderate intensity, Siberian alder might fix 33 ± 31 kg N ha^{-1} yr^{-1} if there had been an alder stand already before the fire, and 91 ± 30 kg N ha^{-1} yr^{-1} if there had been a black spruce forest (Houseman *et al* 2020).

On the one hand, the temperature dependency of BNF might limit annual BNF rates. However, the temperature dependency of BNF by Siberian alder has yet not been analyzed in different environments and may be adapted to the low soil temperatures in permafrost ecosystems. In view of the large influence of BNF on the entire N cycle in permafrost ecosystems, there is a need for research on this topic also with regard to climate warming which might trigger higher BNF rates. In Himalayan alder, BNF per plant increased with plant age, but peaked at the stand level at the age of 15–20 years (Sharma *et al* 2002). It has not been reported whether BNF stops when a critical amount of N has accumulated at the stand level, as previously observed in an *Acacia-Eucalyptus* forest in Australia (Pfautsch *et al* 2009). Thus, the significance

of N_2 fixation of deciduous actinorhizal *Alnus* spp. for the N dynamics of trees, stands and ecosystems in permafrost areas is presently not understood.

The soil N pool can be significantly increased by alder-associated BNF (Nossov *et al* 2011). Also, McCaully *et al* (2021) found first indications for microbially produced NO_3^- from degradation of alder organic matter. In permafrost peatlands of Northeast China, Ramm *et al* (personal communication) found gross ammonification and nitrification rates in soils of alder forests to exceed those of adjacent tree-free peatlands by more than an order of magnitude. Possibly such increases in N mineralization are not only facilitated by BNF-induced N inputs and organic matter with lower C:N ratio. It can be speculated that due to the high energy demand of BNF, the temperature in the surrounding of nodules may increase, thereby generally supporting the N dynamics in permafrost-affected soil. This still needs to be studied both in the laboratory and in the field. Possibly, permafrost alder forests, being widespread in arctic and boreal zonobiomes, and increasing in abundance in the circumpolar permafrost region (Sturm *et al* 2001, Tape *et al* 2006, Lantz *et al* 2010, Frost and Epstein 2014), show a completely atypical N cycle with high mineral N cycling. The latter, combined with anoxic soil conditions, could make such permafrost alder forests hot spots of N_2O emission.

7. Can atmospheric N deposition contribute to release N limitation in permafrost regions?

Atmospheric transportation of reactive N (Nr) compounds ranges in scales up to thousands of kilometers and hence the very remote parts of the world, e.g. the northern circumpolar permafrost region, also receive substantial Nr deposition. The total deposition of Nr involving organic and inorganic forms has increased from less than 0.1 kg N ha^{-1} yr^{-1} in 1860 to the present 0.1–2 kg N ha^{-1} yr^{-1} in the northern circumpolar permafrost region (Galloway *et al* 2004, Dentener *et al* 2006). A further increase of atmospheric deposition in the region is expected during the 21st century due to increased Nr emissions from global and local sources, e.g. arctic shipping, wildfire and exploitation of natural resources (Lamarque *et al* 2005, Dentener *et al* 2006, Peters *et al* 2011). Organic N deposition may contribute globally on average 20%–30% of total deposition, but its rates remain unknown in most of the northern circumpolar permafrost region (Hodson *et al* 2005, Kanakidou *et al* 2016). Inorganic N deposition globally increased by 8% in the recent four decades, which is estimated to be 0.27 kg N ha^{-1} yr^{-1} on average in the Arctic according to the GEOS-Chem Chemical Transport Model (Ackerman *et al* 2019).

A number of N addition experiments have been established to evaluate the effects of atmospheric deposition on arctic and subarctic ecosystems, but most of the experiments are carried out with unrealistically high N addition rates of 40–250 kg N ha^{-1} yr^{-1} and a short duration of 1 year. Very few studies simulate atmospheric deposition with more realistic N addition rates, i.e. $\leqslant$10 kg N ha^{-1} yr^{-1}, for at least 3 years (Gordon *et al* 2001, Madan *et al* 2007, Arens *et al* 2008). The addition of ammonium nitrate (NH_4NO_3) with a rate of 5 kg N ha^{-1} yr^{-1} significantly increased the chlorophyll content of *Polygonum viviparum* leaves at an arctic semi-desert in Svalbard, and altered the CO_2 exchange and normalized difference vegetation index at an arctic tundra in Greenland (Madan *et al* 2007, Arens *et al* 2008). Addition of 5 and 10 kg N ha^{-1} yr^{-1} for 3 years caused N saturation, i.e. the situation that soil N availability exceeds plant and microbial demands, in the arctic semi-desert and tundra, respectively. Addition of NH_4NO_3 with a rate of 10 kg N ha^{-1} yr^{-1} to an arctic tundra heath in Svalbard for 3–8 years led to physiological N saturation of bryophytes as indicated by a strong reduction in nitrate reductase activity and significant changes in the abundance and tissue N content of lichens and bryophytes (Gordon *et al* 2001). The impacts on community composition, nutrient status and NO_3^- assimilation capacity of plants can persist 13–18 years after N addition treatment (Street *et al* 2015). Since the ambient deposition in Svalbard and Greenland is approximately 1 kg N ha^{-1} yr^{-1}, the multi-year N addition experiments indicate that ecosystem structure and function start to change if total deposition exceeds 6–11 kg N ha^{-1} yr^{-1} (Madan *et al* 2007, Kühnel *et al* 2011). The change of ecosystem structure and function can be regarded as an indication of relieving N limitation in the nutrient-poor arctic and subarctic ecosystems. It should be noticed that a lower Nr deposition (e.g. 3–5 kg N ha^{-1} yr^{-1}) may still have the potential to release N limitation in the sensitive ecosystems, e.g. heathland, scrub and tundra, but there is a lack of long-term N addition experiments with such low rates to support the hypothesis so far.

About 80% of the northern circumpolar permafrost region is currently subject to a deposition of less than 1 kg N ha^{-1} yr^{-1}, which cannot alter the N-limited status of arctic and subarctic ecosystems (Dentener *et al* 2006, Forsius *et al* 2010). However, Nr deposition over 3 kg N ha^{-1} yr^{-1} has been observed in large areas of Russia, Alaska and northern Europe (Jónsdóttir *et al* 1995, Woodin 1997). The high Nr deposition in the areas suffering heavy impacts of local pollution and long-range transport from central Europe and North America can greatly release N limitation of ecosystems (up to 10 kg N ha^{-1} yr^{-1}; Woodin 1997). In general, atmospheric N inputs by deposition (0.1–10 kg N ha^{-1} yr^{-1}) and BNF (0.1–25.8 kg N ha^{-1} yr^{-1}; table 2) represent the primary pathways of external N input to the arctic and subarctic ecosystems with low nutrient supply.

The increasing frequency of large fires in the Arctic (Holloway *et al* 2020, McCarty *et al* 2020) might be a decisive factor affecting atmospheric N deposition. In shrubland and forest, often more than 90% of the plant biomass and organic layer is volatilized, e.g. due to pyrodenitrification, while mineral N is accumulating at the site of the fire itself due to the combustion process and mineralization of dead soil microbial biomass (Dannenmann *et al* 2018). Also in dry tundra heath a recent study showed fire-induced mineral N increases (Xu *et al* 2021). However, implications of arctic fires for N biogeochemistry remain severely understudied (McCarty *et al* 2021).

8. Do plants in permafrost ecosystems rather use mineral or organic N?

In general, arctic vegetation is adapted to short growing seasons and relatively low nutrient availability. Due to the perennial nature of numerous arctic plant species, nutrient storage and mobilization minimizes the annual nutrient loss and reduces the demand for annual nutrient uptake required to produce new tissues. In addition, many dominant arctic plant species can use organic N in the soil and atmospheric N_2 either directly, or through root symbiosis with ericoid or ectotrophic mycorrhiza and *Frankia* bacteria symbiosis, as well as their tripartite symbiosis system (plant-*Frankia*-mycorrhiza) (Kielland 1994, Schimel and Chapin 1996, Johansson and Shaver 1999, Dawson 2008, Moore *et al* 2018). These two features constitute a competitive advantage compared to other species that dominantly rely on the annual availability of mineral N (Johansson and Shaver 1999, Rennenberg and Schmidt 2010).

Preferential use of organic N versus mineral N by plants from permafrost ecosystems has been reported in either *in vivo* or *in situ* uptake studies. For instance, a labeling experiment with intact soil cores from arctic tundra in Alaska indicated that roots of woody *Vaccinium* spp. acquired up to three times more N from the amino acid glycine than from NH_4^+ (Walker *et al* 2010). Fungistatic application reduced N acquisition into plant tissue by 30%–40% indicating that the fungal partner of the ericaceous plant roots of *Vaccinium* spp. contributes significantly to N nutrition (Walker *et al* 2010). Higher uptake rates of amino acid compared to mineral N have also been reported for other plant species growing in permafrost ecosystems (Johansson and Shaver 1999, Moore *et al* 2018).

However, application of $^{13}C/^{15}N$-labeled amino acids suggests that some woody shrub species take up amino acid N at least partially *subsequent* to amino acid degradation as indicated by preferential ^{15}N compared to ^{13}C accumulation (Moore *et al* 2018). Apparently, mineral N in the form of NH_4^+ can be taken up by the roots of arctic vegetation.

In the light of low atmospheric deposition but large ammonification rates found across permafrost ecosystems, mineral N taken up by plants very likely originates from N mineralization of organic matter rather than external inputs from the atmosphere. Nonetheless, the significance of this uptake is species-specific and assessing its contribution to plant N nutrition requires further studies.

Despite preferential N accumulation from amino acids compared to mineral N, leaves and roots of numerous species from permafrost ecosystems contain similar amounts of NO_3^- of soil origin as species from temperate and subtropical ecosystems with higher N availability in the soil (Andrews 1986, Schneider *et al* 1996, Simon *et al* 2014, Liu *et al* 2018). Since also the ratios between leaf and soil NO_3^- contents were similar across ecosystems, it has been concluded that despite low NO_3^- availability, NO_3^- uptake capacities in permafrost are similarly high as in low-latitude ecosystems (Liu *et al* 2018). The presence of NO_3^- in both leaves and roots of arctic plant species in the absence of appreciable atmospheric N inputs suggests that at least part of the NO_3^- taken up by the roots is transported to and assimilated in the leaves. *In situ* assimilation in the leaves of arctic plant species has also been assumed from stable isotope analyses of soil and plant NO_3^-, since NO_3^- assimilation by nitrate reductase activity causes an enrichment of ^{15}N and ^{18}O in unassimilated NO_3^- (Liu *et al* 2018). In a modelling approach, Liu *et al* (2018) calculated that NO_3^- uptake can contribute 4%–52% to total leaf N in a range of arctic plant species with particularly low contributions in most ectomycorrhizal plants. This result indicates that mycorrhizal symbiosis is of high significance for the acquisition of DON by plant roots as also observed in previous studies (e.g. Kielland 1994), but that mineral N sources may be particularly important for plants lacking this root symbiosis. It also demonstrates that concluding fluxes of nutrients into plant roots from soil nutrient concentrations can be misleading and that generally NO_3^- cannot be neglected as an N source of arctic plant species.

As a consequence of climate warming, recent studies conducted in alpine ecosystems of the Tibetan Plateau indicated changing soil freeze-thaw front dynamics of permafrost-affected soil in the past decades, i.e. a decreased maximum layer of frozen soil and a delay of beginning and end of the annual frozen period (Wang *et al* 2001, Gao *et al* 2003, Li *et al* 2005). Such changes of soil freeze-thaw dynamics have significantly enhanced concentrations of dissolved organic matter in permafrost-affected soils (Fitzhugh *et al* 2001, Herrmann and Witter 2002, Sharma *et al* 2006). This increase is thought to be a consequence of the disruption of microbial biomass during thawing and the concomitant release of organic N compounds. However, particularly NH_4^+ seems to accumulate in the melt water (Keuper *et al* 2012, Salmon *et al* 2016,

Keuper *et al* 2017, Voigt *et al* 2017a). It is still a matter of debate, if the significant NH_4^+ accumulation in the melt water is a consequence of insufficient root uptake capacity of NH_4^+ compared to DON or of a fast rate of ammonification.

Chang *et al* (2014) reported that simulated freezing-thawing cycles with elevated thawing periods in the Qilian Mountains (northern Tibetan Plateau), China, increased soil DON concentrations 2.42- and 2.82-fold in *Picea crassifolia* stands and stands of alpine shrubs/grasses, respectively, compared to control treatments. Thus, thawing and subsequent mineralization can increase plant-available N at the thaw front of permafrost-affected soils. When N availability was experimentally increased at the freeze-thaw front at 45 cm depth, the roots of several shrubs exclusively present at this soil depth were capable of N uptake between autumn and spring, when aboveground tissue was largely senescent (Keuper *et al* 2017). Nitrogen fertilization of these roots increased aboveground biomass and N content of the deep-rooting plants at a similar magnitude as shallow N fertilization, showing that N taken up by the roots at the thaw front can be transported upwards effectively. Also labeling experiments with ^{15}N–ammonium chloride indicate that the roots of arctic plant species are able to take up mineral N at soil temperatures close to or even below 0 °C (Edwards and Jefferies 2010), but the quantitative significance of this uptake remains to be elucidated. Thus, thawing of permafrost soil by climate warming provides additional N to deep-rooting, N-limited shrubs for growth and development and, therefore, can be assumed to change plant community composition and to counteract increased C loss from thawing permafrost soils due to enhanced biomass production at enhanced N availability (Keuper *et al* 2017). Notwithstanding this, enhanced plant N demand in conjunction with high N losses can also increase N limitation of the vegetation (Kou *et al* 2020).

9. Nitrogen losses induced by mineral N production in permafrost-affected soils

Substantial losses of N via gaseous emissions to the atmosphere through nitrification and denitrification and via leaching of mineral N to water bodies are thought to occur from terrestrial ecosystems that are N-saturated, i.e. where N supply exceeds the immediate needs of plants and microbes (Butterbach-Bahl *et al* 2013). This has led to the assumption of negligible or small gaseous and aquatic N losses from permafrost-affected soils, where mineral N turnover and N pools where supposed to be small and N tightly recycled between plants, microbes and SOM (Shaver *et al* 1992, Buckeridge *et al* 2010, Kicklighter *et al* 2019).

The generally high ammonification and nitrification rates in active layers of permafrost-affected soils

however should also result in significant N losses, even if mineral N limitation prevails due to high microbial immobilization and plant-microbe competition. Furthermore, enhanced N mineralization with warmer temperatures (Salazar *et al* 2020) and N release from permafrost (Keuper *et al* 2012, Voigt *et al* 2017a) will likely increase N losses to the atmosphere and aquatic systems, with important consequences for the ecosystem C balance both on land (via nutrient limitation of plant growth) and in water bodies (via enhanced primary production).

9.1. Gaseous N losses

The gaseous N losses from soils occur mainly in the form of atmospherically inert dinitrogen (N_2) (Scheer *et al* 2020), the strong GHG nitrous oxide (N_2O) (Tian *et al* 2020), and, to smaller extent, the atmospherically reactive gases nitric oxide (NO) (Pilegaard 2013) and nitrous acid (HONO) (Su *et al* 2011, Oswald *et al* 2013). Regarding these gaseous N losses, only those of N_2O have been reported for permafrost-affected soils *in situ*, and even they are understudied compared to gaseous C losses from permafrost regions.

According to a recent review (Voigt *et al* 2020), small N_2O emissions commonly occur from permafrost-affected soils during the growing season, whereas studies on wintertime N_2O exchange in the Arctic are scarce. At the higher end these emissions are comparable to N_2O emissions from tropical or agricultural soils. Nitrous oxide emissions during the growing season tend to be higher from peatlands (median with 25th–75th quartiles: 60 (18–481) μg N m^{-2} d^{-1}) than from upland soils (34 (6–170) μg N m^{-2} d^{-1}) or from wetlands with low organic matter content (19 (-8–19) μg N m^{-2} d^{-1}). Higher emissions occur from bare soils (455 (165–779) μg N m^{-2} d^{-1}) compared to vegetated soils (30 (6–89) μg N m^{-2} d^{-1}) (Voigt *et al* 2020). As typical for soil N_2O fluxes (Butterbach-Bahl *et al* 2013), N_2O emissions from permafrost-affected soils show high spatial and temporal heterogeneity (Voigt *et al* 2020), with high emissions e.g. from bare peat surfaces on permafrost peatlands, reaching up to over 6000 μg N m^{-2} d^{-1} during the growing season (Repo *et al* 2009, Marushchak *et al* 2011). On the other hand, high N_2O emissions have recently been confirmed from Alaskan tussock tundra on a landscape scale with air-borne measurements (Wilkerson *et al* 2019), suggesting widespread emissions across the landscape. Since N_2O is produced during mineral N transformation processes (Butterbach-Bahl *et al* 2013), substantial emissions confirm significant microbial production and turnover of mineral N.

Due to lack of direct *in situ* measurements of N_2, NO and HONO from permafrost-affected soils, we can only speculate about their occurrence. But, since these gases are produced by the same mineral N transformation processes as N_2O (Butterbach-Bahl

et al 2013, Oswald *et al* 2013, Pilegaard 2013), soils with substantial N_2O emissions (permafrost peatlands, bare soils) can be considered as the most potential sources for the other gaseous N forms as well. Scheer *et al* (2020) estimated that at the global scale N_2O comprises approximately 8% (6%–11%) of the terrestrial denitrification flux. Assuming that on average permafrost-affected soils emit 288 μg N m^{-2} d^{-1} in the growing season (Voigt *et al* 2020), this would equal to about 0.3 kg N_2O–N ha^{-1} growing season^{-1} of 100 d, or eventually 3 kg N ha^{-1} growing season^{-1} of total denitrification as estimated based on the global ratios of N_2O to total denitrification provided by Scheer *et al* (2020). There is additional direct evidence for N_2 release from high-N_2O-emitting subarctic peat soils as a result of complete denitrification, i.e. N_2O reduction to N_2, from application of the acetylene inhibition method (blocking N_2O reduction to N_2) *in vitro* (Palmer *et al* 2012) and from stable isotope studies of N_2O in the pore gas *in situ* (Gil *et al* 2017). Production of NO was observed in a laboratory incubation conducted with Alaskan tundra soils, with strong positive dependence on mineral N content (Yonemura *et al* 2019).

9.2. Aquatic N losses

Permafrost landscapes are characterized by low hydrological landscape conductivity, shallow flow paths of water (Sjöberg *et al* 2020), and high abundance of water bodies and water-logged soils (Vonk *et al* 2015). The magnitude, timing and composition of lateral N losses from land to aquatic systems will change with permafrost thaw processes such as thermokarst erosion (Turetsky *et al* 2019) and active layer deepening (Biskaborn *et al* 2019), combined with changing precipitation patterns (Bintanja and Andry 2017). The particulate organic N transport by rivers to the Arctic Ocean has been estimated at 695 Gg N and is associated with dissolved N export (DON and mineral N) of equal magnitude (McClelland *et al*, 2016), and will likely increase in the future with permafrost thaw (Connolly *et al* 2020).

There are differences in the magnitude and composition of dissolved N losses by leaching between soil types and layers. Organic soils have a higher potential for overall N leaching losses (DON and mineral N) than mineral soils (Wickland *et al* 2018, Fouché *et al* 2020). In organic soils, N leaching may be dominated by DON (Wickland *et al* 2018, Fouché *et al* 2020) and NH_4^+ (Fouché *et al* 2020). Leaching of NO_3^- may be more important for well-drained mineral soils and is expected to increase with deepening active layers and exposure of mineral soil layers in thermokarst features (Harms and Jones 2012, Harms *et al* 2014). This can have great implications for plant nutrition (see section 8).

Interestingly, the content of leachable N is typically higher in permafrost layers compared to active layers (Keuper *et al* 2012, Beermann *et al* 2017,

Wickland *et al* 2018, Fouché *et al* 2020), suggesting higher lateral N losses with progressing permafrost thaw (Connolly *et al* 2020). Enhanced and persistent mineral N losses via leaching have been observed from permafrost thaw slumps (Bowden *et al* 2008, Abbott *et al* 2015) and thermokarst gullies (Harms and Jones 2012). Elevated mineral N concentrations observed in arctic rivers (Jones *et al* 2005, Bowden *et al* 2008) indicate that mineral N losses from permafrost-affected landscapes are increasing.

Besides dissolved N discharge from thermokarst features, substantial N inputs into aquatic systems occur with direct sediment losses associated with wildfires (Abbott *et al* 2021) and fluvio-thermal erosion and thermokarst along river banks (Kanevskiy *et al* 2016, Fuchs *et al* 2018) and coast-line (Günther *et al* 2013). In addition to permafrost thaw processes, longer thaw seasons will likely enhance leaching of mineral N through continued ammonification and nitrification in fall with little N uptake by plants or microbes (Treat *et al* 2016b).

10. Mineral N cycling matters: a revised paradigm, persisting knowledge gaps and recommendations for future studies

This review demonstrates that the paradigm of a predominance of organic N cycling in permafrost-affected soils cannot be confirmed by gross N turnover data published during recent years (see figure 1(b)). In contrast, mineral N cycling is present and important in the active layer of permafrost-affected soils to a similar extent as in temperate or even tropical soils and its main processes ammonification and nitrification are similarly dependent on SOC and TN. This is because functional limitations of SOM decomposition, N mineralization and mineral nutrient cycling are largely released upon permafrost thaw so that soil microbial N cycling does not stop at the level of DON in these cold environments. Considering the presence of an abundant microbial community involved in mineral N cycling both in active and permafrost layers, the large C and N stocks of permafrost-affected soils and the fact that the main functional limitation for N cycling is exerted by frozen soil, mineral N turnover might increase in a warmer future. In a changing Arctic, there may be increasing priming and N mining due to increased rooting and exudation, mobilizing additional SOM/SON and accelerating N cycling even more. Permafrost retreat thus bears a high potential not only for C but also for N mineralization even in soils with high C:N ratios.

Our meta-analysis showed that gross ammonification in permafrost ecosystems can be high enough to allow for significant mineral N nutrition of plants which might be especially relevant when no ectomycorrhiza are present. As plants compete strongly for both organic and inorganic N forms with microbes

in permafrost areas, net N turnover rates tend to be around zero and do not depict which processes are taking place and at which magnitude. Thus, in fact, net rates might be particularly misleading with respect to permafrost-affected soils, and their widespread use can explain how the old paradigm has established in the first place. While net N mineralization was found to decrease with latitude (and altitude) (Liu *et al* 2016, 2017), this is not indicated for gross ammonification and nitrification. High depolymerization rates do not only question the paradigm that depolymerization is the bottleneck of N cycling, but also—together with high ammonification, nitrification and nitrification:ammonification ratios—suggest that N limitation is not particularly pronounced in permafrost-affected soils. Among the wide and diverse range of different permafrost ecosystems, mineral N cycling and associated N losses appear to be especially important in N-rich organic soils, when plant cover is absent and when impacted by cryoturbation or erosion (such as thermokarst). However, more data are needed to reveal influences of different ecosystem types on gross N turnover as no significant differences were found (not even when comparing unvegetated to vegetated ecosystems). With an increasing amount of available data, geographical patterns should be analyzed in more detail in future review articles.

Currently, the process-based and quantitative understanding of N cycling in permafrost-affected ecosystems is still limited by a lack of experimental evidence on gross N turnover rates (particularly on gross protein depolymerization), with regard to seasonal dynamics and dynamics in the vertical soil profile (e.g. along the freezing/thawing front and water table) (table 3). As soils freeze from the surface in autumn/winter there is a phase where the upper part of the active layer is frozen, but is underlain by a non-frozen part. In contrast to temperate soils, below this non-frozen layer there is the permanently frozen core. Currently, nothing is known about N turnover and gas accumulation between the two frozen layers, while it has been shown that these soil conditions are relevant for CH_4 release (Mastepanov *et al* 2008, Pirk *et al* 2015, Zona *et al* 2016). Nitrogen turnover processes in frozen soil itself are also generally unknown but could matter as few studies reported for non-permafrost soils. A methodological framework to study N transformation processes in frozen soil is urgently needed to gain insight into annual N cycling in permafrost-affected soils, in particular in tundra uplands and the high arctic. Furthermore, the microbiome performing ammonification is not well-known for permafrost-affected soils. Also processes such as anammox and DNRA are severely understudied (table 3).

There is increasing evidence that arctic plants in addition to DON can use also mineral N forms

Table 3. Key findings of this review, permafrost (PF) research gaps referring to the different research topics explored in this review, and recommendations for future studies.

Research topic	Key findings of this review	Research gaps	Recommendations for future studies
Soil N stocks and gross N turnover	• Gross ammonification and nitrification rates are of similar magnitude in active layers in PF systems compared to temperate/tropical systems. • Thawing of PF soil releases functional limitations for N cycling. • Gross mineral N cycling across PF ecosystems depends on SOC and TN. • High gross nitrification rates question N limitation. • Depolymerization is likely not the rate-limiting step for ammonification as depolymerization rates by far exceed ammonification rates, but scarce data. • Reported N mineralization rates allow for significant plant mineral N nutrition. • Net rates for microbial N turnover are close to zero, and, thus, do not reflect the high dynamic of microbial N turnover in PF-affected soils.	Seasonality remains uncertain as only snapshot studies available for gross rates, no wintertime fluxes. Improved quantification of additional N inputs to ecosystems due to PF thawing. Gross N turnover in different PF ecosystem types. Gross N turnover dynamics in the vertical soil profile and in frozen soil. Landscape-scale dynamics of gross N turnover. Temperature sensitivity of gross N turnover. Importance of depolymerization as a control of ammonification, rates of anammox/DNRA (difficult quantification). Re-assessment/comparison of uncertainties associated with methodologies for gross and net rate determination.	^{15}N pool dilution studies *in situ* with at least seasonal temporal resolution including winter, linking measurement with biogeochemical modelling studies. Differentiation of N sources in thawing PF layers by ^{15}N tracing studies. *In situ* studies that cover a wide range of PF systems. Depth profiles of N turnover based on *in situ* incubations, method development for frozen-soil studies. Landscape transect sampling, testing indicators of gross N turnover. Field warming studies and laboratory incubation studies on temperature effects on gross N turnover. Methodological framework to quantify these processes. Experiments evaluating uncertainties of methodologies.
Microbiome involved in N turnover	• Nitrifiers and denitrifiers are abundant in PF-affected soils.	Microbiome involved in ammonification, anammox, DNRA.	Linking molecular microbiome with biogeochemical process studies, assessing the role of C:N:P stoichiometry.
BNF	• BNF by bryophytes and lichens might significantly contribute to N input in PF systems, but difficult upscaling (possibly leads to overestimation). • Very high BNF potential of N_2-fixing shrubs.	Landscape-scale N_2 fixation, considering seasonal variability. Relationships between N_2 fixation and N mineralization Temperature dependency of N_2 fixation. Symbiotic N_2 fixation by shrubs hardly quantified.	Linking molecular studies with biogeochemical quantification and remote sensing studies to bridge scales. $^{15}N_2$ tracing studies to assess conversion to soil mineral N. Laboratory parameterization studies Study of fixation rates and distribution of alders in PF systems.

(Continued.)

Table 3. (Continued.)

Research topic	Key findings of this review	Research gaps	Recommendations for future studies
Atmospheric N deposition	• Rates overall seem still low in the Arctic except for fire-induced deposition.	Few measurements available	Network of N deposition measurements in the Arctic
Plant N uptake	• Arctic plants in addition to DON can also use mineral N, in particular in absence of mycorrhiza.	Actual uptake rates uncertain	Plant mineral N uptake rates in intact PF plant-soil systems.
Gaseous N losses	• Relatively high N$_2$O emissions from arctic soils support the occurrence of large mineral N turnover rates.	Annual fluxes for most representative PF systems considering the high spatiotemporal variability.	Continuous, year-round flux measurements at research stations with dedicated infrastructure.
		HONO, N$_2$, NO fluxes unknown	First field measurements urgently needed (but challenging methodology).
		Importance of GHG fluxes from surface waters unknown	Measurements of GHG fluxes from water bodies combined with catchment-scale studies on nutrient fluxes.
Aquatic N losses	• River water concentrations indicate a significant role of mineral N leaching.	Information on leaching at site scale missing	Continuous N concentration measurements in creeks/rivers, assessment of lateral N leaching at site scale.

for growth, particularly those which are not in association with mycorrhizal fungi and *Frankia* bacteria symbioses. Plants relying on mineral N forms may even have a competitive advantage in a future warmer world, when permafrost thawing and climate warming trigger the availability of relatively high amounts of NH_4^+ and NO_3^- by enhanced microbial mineral N cycling. The significance of higher plants and their interaction with rhizospheric microorganisms for N cycling in permafrost ecosystems are still largely unexplored. Especially how BNF influences N mineralization is not clear. There is a research gap concerning the physiological, biochemical and molecular mechanisms that allow BNF and plant root N uptake at freezing temperatures. Studying interactions of plant physiology with soil biogeochemistry will be decisive for predicting climate change effects on nutrient cycles and GHG balances in warming permafrost ecosystems, especially with regard to alder shrubs (table 3).

The outlined high importance of mineral N cycling in permafrost-affected soils suggests that also gaseous N losses may be higher as previously assumed, but so far only fluxes of N_2O have been studied, while reports on other forms of gaseous N losses from permafrost-affected soils (e.g. HONO, NO, N_2) are almost completely missing. Findings of high N_2O emissions from permafrost should not be neglected as they are potentially relevant as another feedback mechanism to climate change. Already today, permafrost-affected soils emit 0.17–1.3 Tg N_2O–N yr^{-1} (Voigt *et al* 2020), i.e. up to 20% of the N_2O emissions from soils under natural vegetation worldwide (6.6 Tg N_2O–N yr^{-1}; Ciais *et al* 2013). It has been estimated that between 7% and 15% of organic C might be released from permafrost in this century (Schuur *et al* 2015). When analogously assuming that ca. 10% of the organic N store will be released until 2100, and only 1% thereof will be emitted as N_2O (conservative estimate as 1% is the default N_2O emission factor of the Intergovernmental Panel on Climate Change for N mineralized from mineral soils; IPCC 2006), this would mean additional 67 Tg N_2O–N until 2100. This translates into ca. 0.8 Tg N_2O–N yr^{-1} which would approximately double current average emissions from permafrost, with a huge uncertainty of this estimate. Regarding aquatic losses, the lateral transport of N (e.g. NO_3^- leaching through draining of thermokarst) is an important research field to gain insight into possible nutrient shifts from terrestrial to aquatic ecosystems. Also, arctic fires might not only cause N volatilization and re-deposition at large scales, but accelerate permafrost thaw and possibly promote mineral N cycling at large scales, however this has been rarely assessed for different permafrost ecosystems.

Consequently, the research gaps on permafrost N cycling (table 3) need to be addressed by interdisciplinary studies involving atmospheric/soil physicists, biogeochemists, microbiologists, hydrologists and plant physiologists to investigate N cycling in intact plant-soil-microbe systems at the landscape scale, thereby considering plant-soil-microbe C:N:P interactions, seasonal dynamics, and vertical soil profile dynamics. Such studies will be a prerequisite for better assessing permafrost nutrient climate feedbacks.

Data availability statement

Any data that support the findings of this study are included within the article.

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