Tree species and moisture effects on soil sources of N_2O: Quantifying contributions from nitrification and denitrification with ^{18}O isotopes

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[1] Nitrous oxide (N_2O) is an important greenhouse gas and participates in the destruction of stratospheric ozone. Soil bacteria produce N_2O through denitrification and nitrification, but these processes differ radically in substrate requirements and responses to the environment. Understanding the controls over N_2O efflux from soils, and how N_2O emissions may change with climate warming and altered precipitation, require quantifying the relative contributions from these groups of soil bacteria to the total N_2O flux. Here we used ammonium nitrate (NH_4NO_3, including substrates for both processes) in which the nitrate has been enriched in the stable isotope of oxygen, ^{18}O, to partition microbial sources of N_2O, arguing that a molecule of N_2O carrying the ^{18}O labeled will have been produced by denitrification. We compared the influences of six common tree species on the relative contributions of nitrification and denitrification to N_2O flux from soils, using soils from the Siberian afforestation experiment. We also altered soil water content, to test whether denitrification becomes a dominant source of N_2O when soil water content increases. Tree species altered the proportion of nitrifier and denitrifier-derived N_2O. Wetter soils produced more N_2O from denitrification, though the magnitude of this effect varied among tree species. This indicates that the roles of denitrification and nitrification vary with tree species, and, that tree species influence soil responses to increased water content.

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1. Introduction

[2] N_2O is a major greenhouse gas and also participates in the destruction of stratospheric ozone [*Crutzen*, 1981]. Denitrification and nitrification are the main biological processes leading to N_2O formation and emission from the soil [*Davidson*, 1991]. Because denitrification is favored when soils are moist and anaerobic, whereas nitrification is favored under more mesic to xeric conditions, understanding the relative contributions of each process to total N_2O emission is critical for modeling and predicting changes in N_2O fluxes under varying environmental conditions, including altered precipitation patterns and soil moisture regimes. In the past, soil sources of N_2O have been identified using selective inhibitors, sterilization, or by adding substrates [*Davidson and Schimel*, 1995; *Stevens et al.*, 1997]. Another potential way to identify the processes producing N_2O is to measure the natural abundance stable isotope composition of N_2O [*Yoshida*, 1988; *Yoshinari*, 1990]. The isotopic composition (i.e., ratios of $^{15}N/^{14}N$ and $^{18}O/^{16}O$) of denitrifier-derived N_2O often differs from that of nitrifier-derived N_2O, especially under laboratory conditions [*Kim and Craig*, 1990; *Webster and Hopkins*, 1996]. However, using this difference to distinguish the biological processes underlying production of N_2O in the field is problematic, owing to uncertainties with the level of fractionation by denitrification and nitrification [*Pérez et al.*, 2000] as well as numerous other sources of variation in the isotopic composition of N_2O [*Menyailo et al.*, 2003; *Schmidt et al.*, 2004]. Potentially, more information can be received if the positioning of ^{15}N within the N_2O molecule is considered [*Stein and Yung*, 2003; *Sutka et al.*, 2006], but also here there are many uncertainties.

[3] Isotope tracer approaches have the advantage that biological kinetic fractionations are much smaller compared to the isotopic signature of enriched substrates, and so fractionations become negligible sources of variation in tracer studies [*Panek et al.*, 2000]. Several attempts have been undertaken to distinguish the sources of N_2O using ^{15}N [*Panek et al.*, 2000; *Baggs et al.*, 2003]. However, after several hours of $^{15}NH_4^+$ application, nitrification produces $^{15}N\text{-}NO_3^-$, causing N_2O formed by denitrifiers to also be enriched and therefore mistakenly considered to be nitrifier-derived. Similarly, if $^{15}NO_3^-$ is applied, dissimilative reduction of nitrate to ammonium (DRNA) can enrich NH_4^+, and N_2O produced from nitrification will be enriched but incorrectly considered to be denitrifier-derived.

[4] Using oxygen isotopes in NO_3^- could address some of these issues. The oxygen atom in nitrous oxide produced

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through nitrification is derived from either atmospheric O_2 or from H_2O [*Dua et al.*, 1979; *Hollocher et al.*, 1981; *Andersson and Hooper*, 1983; *Kumar et al.*, 1983; *Ostrom et al.*, 2000]. Thus we can expect that oxygen in nitrifier-derived N_2O should reflect the isotopic composition of oxygen of both O_2 and H_2O. By contrast, in denitrification, oxygen in the N_2O product originates mostly from the oxygen in NO_3^-, with minor contributions from soil H_2O [*Tilsner et al.*, 2003]. Using isotope-ratio mass spectrometry, it should be possible to measure the contribution of each process to total N_2O efflux, even if the impact of one of the processes is small. *Wrage et al.* [2005] reported the application of enriched ^{18}O-H_2O in combination with ^{15}N to distinguish the sources of N_2O, but to our knowledge no studies have reported the use of enriched ^{18}O-NO_3^- for this purpose.

[5] To test the applicability of ^{18}O for distinguishing N_2O sources, we used soils from a Siberian afforestation experiment, in which six common Siberia tree species were planted and allowed to grow for about 30 years on initially homogeneous soil. In this experiment, we have already documented tree species effects on denitrification and net nitrification potential [*Menyailo et al.*, 2002b]. Thus differences between tree species in the relative contributions of denitrification and nitrification to soil N_2O production can be expected. Furthermore, we incubated soil samples collected under different tree species at two moisture levels to additionally alter the proportion of denitrifier- versus nitrifier- derived N_2O and to test whether the isotopic signatures of oxygen in N_2O will be more enriched due to hypothesized increase of denitrifier-derived N_2O at higher soil moisture. The aims of this work were (1) to assess the use of ^{18}O labeling in NO_3^- to distinguish the sources of N_2O, (2) to clarify if tree species alter proportion of denitrifier- versus nitrifier-derived N_2O and (3) to estimate the effect of altered soil moisture on the sources of N_2O and to check if these effects depend on tree species.

2. Methods

2.1. Sites and Soil Samples

[6] The research plots are located 50 km Northwest from Krasnoyarsk and were established by the Laboratory of Soil Science of the Institute of Forest, Siberian Branch of the Russian Academy of Sciences [*Menyailo et al.*, 2002a]. The upper 0–50 cm of soil of a 1.5-ha area were removed, mechanically homogenized to minimize vertical and spatial heterogeneity of chemical, physical and biological properties, and subsequently returned to the site prior to experimental planting. In 1971–1972, 2- to 3-year-old seedlings of spruce (*Picea abies*), birch (*Betula pendula*), Scots pine (*Pinus sylvestris*), aspen (*Populus tremula*), larch (*Larix sibirica*) and Arolla pine (*Pinus cembra*) were sown into individual pure species plots, each occupying 2400 m^2. An area of 9600 m^2 was left for grassland as a control, and the soil under grass was not mechanically homogenized. The region is characterized by continental climatic conditions with average rainfall 500 mm yr^{-1}, average daily summer temperature of 20°C (at 12:00), depth to permafrost 70–170 cm, and soil temperature to 20 cm depth in winter $-4°$ to $-14°$, in summer 10° to 12°. The soil is the gray forest type according to the Russian Soil Classification System and Greyzem according to *Food and Agriculture Organization (FAO)* [1990]. In August 2001, each plot was subdivided into three parts: A, B and C (as in work by *Menyailo et al.* [2002a]). From each subplot, two trees were randomly chosen and four soil samples (0–10 cm) were taken at a distance of 50 cm from the stem of each tree in cardinal directions. In the grassland plot, three subplots (each of 2 m^2) were chosen along the forest plantation; at each subplot six soil samples were taken from the 0–10 cm depth. Soil samples from each subplot were mixed. The total number of soil samples was 21: six species plus grassland by three subplots. All soil samples were air-dried and sieved (2 mm).

2.2. Incubation Experiments

[7] The first experiment was carried out with one soil sample collected from grassland to determine the maximum instantaneous ^{18}O enrichment of N_2O during denitrification, and thereby to provide the isotopic end-member for calculations of the relative contributions of nitrification and denitrification. Fifteen grams of each of four subsamples were placed in 250-mL glass flasks, moistened with distilled water and preincubated at 25°C for 3 days to initiate microbial activity and to reduce the concentrations of background NO_3^- and NH_4^+. After that, NH_4NO_3 was added to each subsample in 1 mL of water, enough to bring soil water holding capacity (WHC) to 90%. Care was taken to moisten soils evenly. The rate of N addition was 500 mg N-NH_4NO_3 kg^{-1} dw; the target enrichment for ^{18}O-NO_3^- was 1.6 atom%. Half of the subsamples received 25 mL (10% v/v) of C_2H_2, to inhibit N_2O-reductase and nitrification.

[8] The samples were incubated for 2 days at 25°C. After 2, 4, 8, 16, 26, 36 and 48 hours, the headspace of each flask (5 mL) was sampled for the analysis of N_2O and CO_2 using a gas chromatograph (Agilent 6890), equipped with electron capture detector (ECD ^{63}Ni), flame ionization detector (FID) and in-line methanizer. Results were recorded as mg N-N_2O kg^{-1} and mg C-CO_2 kg^{-1}. Additionally, 1 mL of the headspace was taken and injected in 20-mL glass vials filled with helium for later $\delta^{18}O$-N_2O measurements. The storage was necessary because the isotope-ratio mass spectrometer (IRMS) operates in 1000 times narrower concentration ranges than the gas chromatograph, and the concentration of N_2O injected into the IRMS should be known before analysis for dilution, if necessary.

[9] Because the flasks were not opened between sampling times, the measured isotopic values are cumulative, including probably some nitrifier-derived N_2O due to aerobic conditions (even in the presence of C_2H_2). We expected that at the end of incubation, when more of the O_2 has been reduced, the conditions will be more anaerobic and the denitrifier-derived N_2O will dominate. Therefore the instantaneous enrichment of $\delta^{18}O$-N_2O was estimated and a further assumption was made that $\delta^{18}O$ values of N_2O derived from the soil at the latest stage of incubation with C_2H_2 corresponds to 100% denitrifier-derived N_2O. The maximum instantaneous enrichment of oxygen in N_2O evolved was estimated using the mass balance equation for all sampling points, beginning with the second (4 hours),

$$AP = (M_2 \times AP_2 - M_1 \times AP_1)/(M_2 - M_1), \qquad (1)$$

where M_1 and M_2 are concentrations of N_2O in the flask at a given time and subsequent time, and AP_1 and AP_2

are the ^{18}O enrichment in N_2O at a given time and subsequent time.

[10] The second incubation experiment was carried out using all 21 soil samples, using the protocol described above, with the difference that instead of the C_2H_2 treatment, all soils were incubated at two levels of soil moisture: 30% of WHC (low moisture) and 90% of WHC (high moisture). The headspace from the flasks was sampled after 10, 26, 50, 74 and 142 hours of incubation.

2.3. Isotopic Measurements in N_2O

[11] The ratios of the stable isotopes $^{18}O/^{16}O$ in N_2O emitted from soils were determined using an on-line GC-IRMS system, consisting of a trace gas cryogenic preconcentration device (PreCon, ThermoQuest), gas chromatograph (ThermoQuest) with Plot Q capillary column (0.32 mm × 30 m), and an isotope-ratio mass spectrometer (ThermoQuest Delta^{PLUS}).

[12] The ratios of masses 46:44 in N_2O samples were measured and used to estimate ratios of $^{14}N_2^{18}O/^{14}N_2^{16}O$. We used N_2O as reference gas (99.9990%, Linde). The $\delta^{18}O$ in N_2O were referenced to another N_2O standard provided by T. Pérez (UC Irvine). Using the natural abundance variations of the isotopic composition of N_2O is seriously complicated by the lack of an international standard for isotopes in N_2O. However, because our studies used artificially enriched work, an exact calibration to natural abundance values is not necessary.

2.4. Statistical Data Analysis

[13] The first incubation experiment was performed with two duplicates for one soil sample. Total number of flasks is four (one sample, with and without C_2H_2, two replicates). The second incubation experiment was done without duplicates for each soil sample, thus yielding three replicates (three soil samples for one tree species). The total number of flasks for the second experiment was 42: three samples for each of the six species and the grassland by two levels of soil moisture). For rates of CO_2 and N_2O production, the effects of tree species, soil moisture, and their interactions were determined with two-way ANOVA. The low moisture treatment resulted in much less N_2O production, not always yielding enough for isotopic analysis (thus some time points were missing). Because of an incomplete data set for ^{18}O-N_2O for the low soil moisture treatment, and because our primary interest was the effects of species and moisture, we averaged ^{18}O-N_2O values throughout the incubation and performed two-way ANOVA with species and moisture as main effects. The effects of species and moisture on ^{18}O in N_2O were calculated with two-way ANOVA. Where the main effect was significant, LSD post hoc comparisons were used to determine significant differences between treatments. We considered the effect significant at $P < 0.05$. All statistics were carried out with the statistical package STATISTICA (5.0 for Windows) [*StatSoft*, 1997].

3. Results and Discussion

3.1. Determination of the Maximum ^{18}O Enrichment During Denitrification in Grassland

[14] The maximum enrichment of ^{18}O-N_2O during denitrification was 1.33 atom% ^{18}O-N_2O. The production of CO_2 was linear during 48 h of incubation, suggesting no limitation of soil heterotrophic microorganisms, including denitrifying bacteria, by C availability in grassland (Figure 1a). The application of C_2H_2 had no effect on CO_2 production, indicating that it is unlikely that soil microorganisms used C_2H_2 as additional C source. The N_2O production was not affected by C_2H_2 during the first 8 hours of incubation, but afterwards the flasks without C_2H_2 accumulated significantly less N_2O, indicating that N_2O-reductase activity increased through time (Figure 1b).

[15] The cumulative and calculated instantaneous ^{18}O-N_2O values are shown in Figures 1c and 1d. In both treatments (with and without C_2H_2), N_2O became enriched in ^{18}O throughout the incubation, indicating an increase in the relative proportion of denitrifier-derived N_2O. This is likely due to depletion of O_2 in the flasks, creating anaerobic conditions favorable for denitrification. This was even more pronounced for instantaneous values of ^{18}O-N_2O (Figures 1c and 1d), which show the actual temporal shift in the relative contributions of denitrifiers and nitrifiers to N_2O production. The instantaneous ^{18}O-N_2O values without C_2H_2 were slightly higher at the end of incubation than in the flasks with C_2H_2, likely as a result of N_2O-reductase activity. The calculation of instantaneous ^{18}O-N_2O relies on N_2O concentrations (see equation (1)). Thus, by reducing N_2O concentrations in the flasks with little effect on the isotopic composition of N_2O (in enrichment studies such as this one, the fractionation has a negligible effect on atom% values), the increasing activity of N_2O reductase contributed to higher instantaneous ^{18}O-N_2O values. Because natural abundance of ^{18}O-N_2O is about 0.2 atom%, corresponding to 100% of nitrifier-derived N_2O (no ^{18}O enriched substrate is incorporated into N_2O via nitrification), the contribution of each of the two processes to N_2O production can be calculated using the equation shown in Figure 2.

3.2. Species Effects on CO_2 and N_2O Fluxes

[16] In the second experiment, soil samples under different tree species and grassland were incubated at two levels of soil moisture. CO_2 production was measured to determine whether low moisture limited microbial activity and whether the water solution was uniformly distributed over the entire soil sample at low moisture treatment. CO_2 and N_2O production were more or less linear throughout the incubation period. Overall, species strongly affected CO_2 production (Figure 3), mostly owing to very high rate in grassland ($P < 0.001$). Soil moisture had no effect on CO_2 production rate indicating no limitation of soil microorganisms by water. Increasing soil water content enhanced N_2O production by a factor of 10–100 ($P < 0.001$, Figure 4). The effect of tree species on net N_2O production (main effect, $P = 0.010$) depended on soil moisture (species × moisture interaction, $P = 0.013$). At low soil moisture, species had no effect on net N_2O production. At high soil moisture, soil beneath aspen produced more N_2O than soils beneath Scots pine ($P = 0.028$) and larch ($P = 0.047$). This is important for predictions of future N_2O efflux from the Siberian forest ecosystems in response to changing tree species composition. Both spruce and larch forests cover large territories in Russian Siberia. If these coniferous species are replaced by hardwood aspen, as predicted in response to global climate change [*Pastor and*

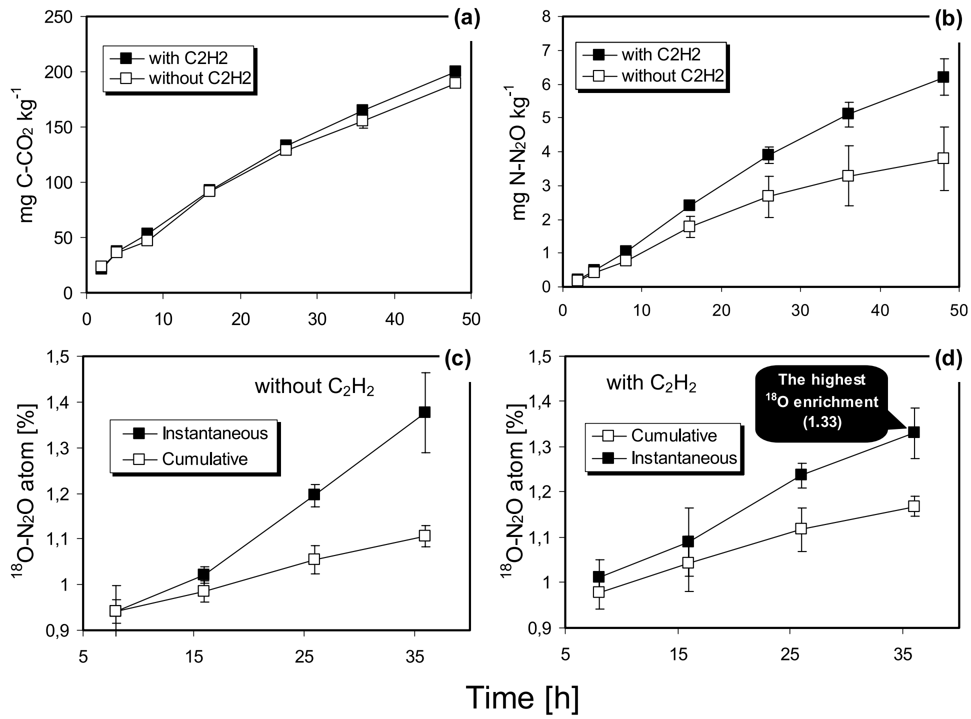

Figure 1. Accumulation of (a) CO_2, (b) N_2O, and the cumulative and instantaneous ^{18}O enrichment in N_2O (c) without and (d) with C_2H_2 in the incubation experiment with soil samples from grassland (n = 2). Highlighted is the highest instantaneous enrichment (1.33) of ^{18}O-N_2O observed during denitrification, which was used as the isotopic end-member for denitrifier-derived N_2O.

Post, 1998], the capacity of Siberian forest soils for N_2O production might increase.

3.3. Enrichment ^{18}O-N_2O Values During Incubation

[17] At the high level of soil moisture, ^{18}O-N_2O varied between 0.74 and 0.95 atom% at the beginning of incubation, becoming enriched to 0.96 and 1.25 atom% at later stages of incubation (Figure 5). As in the grassland (Figures 1c and 1d), in the forests the relative contribution of denitrifiers to total N_2O efflux increased and the contribution of nitrifiers decreased throughout the incubation. Considering the weighted average for all time points together, tree species affected ^{18}O enrichment: the effect was significant ($P < 0.001$) mostly owing to spruce, in which the ^{18}O composition of N_2O was 0.1-0.2 atom% lower compared to all other tree species ($P < 0.050$). Thus, at high soil moisture, soil under spruce had the lowest ^{18}O-enrichment and thus probably the highest contribution of nitrification to total N_2O production (Figure 6). For comparison, the ^{18}O-N_2O values for the low moisture treatment are also presented here (Figure 6). At low moisture, tree species also affected oxygen enrichment in N_2O. Aspen had higher ^{18}O-N_2O values than spruce (P = 0.011), Arolla pine (P = 0.008) and birch (P = 0.037). Also, grassland had higher ^{18}O-N_2O values than Arolla pine (P = 0.045).

[18] While tree species differed in ^{18}O enrichment ($P <$ 0.001), soil moisture also had a large effect on ^{18}O-N_2O ($P <$ 0.001) (Figure 6). Increased soil moisture caused an increase ^{18}O-N_2O enrichment under all tree species by 120–165%, likely because wetter soils enhanced denitrification and decreased nitrification, increasing the contribution of denitrifiers to total N_2O efflux.

[19] The difference in ^{18}O enrichment between low and high moisture levels was species-dependent (interaction species versus moisture $P < 0.001$), at least in part because differences among tree species were larger at low moisture. This is the first evidence that the relative importance of the processes responsible for N_2O efflux differs under different tree species, and that they respond in distinct ways to altered precipitation patterns and soil moisture regimes. Using the equation given in Figure 2, the proportions of denitrifier- and nitrifier-derived N_2O were calculated for low moisture

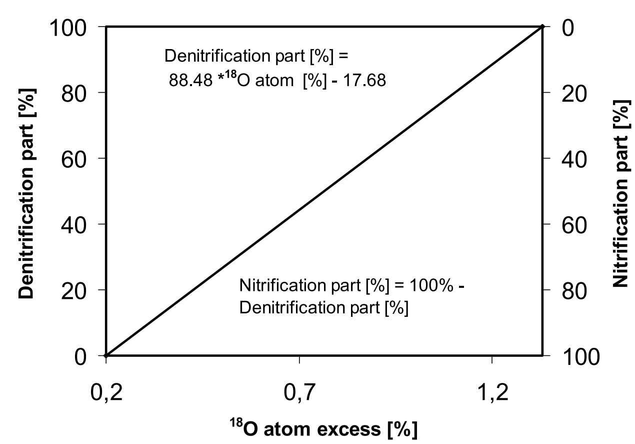

Figure 2. Conceptual scheme for estimating the contribution of nitrification and denitrification to total N_2O efflux. The values are expected to range between 0.2 atom%, when contribution of denitrification is 0%, and 1.33 atom% when contribution of denitrification is 100%.

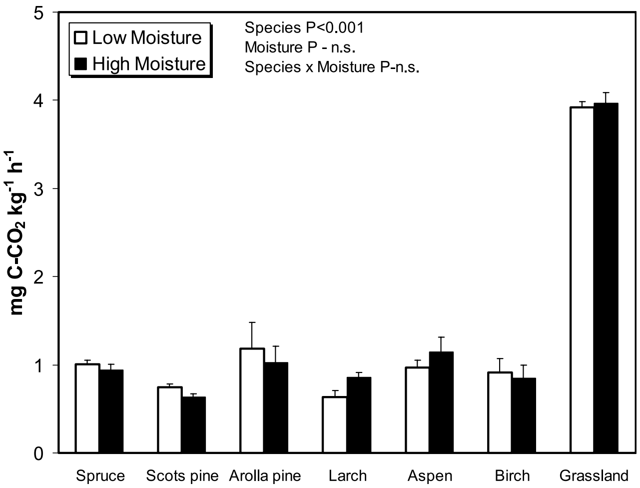

Figure 3. Rate of CO_2 production under 6 tree species and grassland during 144-hour incubation at two levels of soil moisture (low: 30% of WHC, and high: 90% of WHC). We found no effect of soil moisture but strong effect of species, mostly due to high rates of CO_2 production in grassland (n = 2).

and for high moisture at the beginning and at the end of the incubation (Figure 7). At low soil moisture, the contribution of denitrification varied between 7% and 38% depending on species. With increasing soil moisture, the contribution from denitrification increased to 52–63% at the beginning of the incubation, and, with the depletion of O_2 during the course of the incubation, increased further to 75–93%. With increasing soil moisture and net N_2O efflux, denitrification becomes the main source of N_2O. At the same time, only at high soil moisture did species significantly affect net N_2O production. Varying contributions of denitrifier-derived N_2O under different species does not appear to explain species effects on N_2O efflux, because species that differed in net N_2O production at high moisture (Figure 4) did not differ in the relative contributions of denitrification and nitrification to total N_2O production at high moisture (Figures 6 and 7). Alternatively, aspen soils may support higher rates of N_2O reductase activity than soils under Scot pine and larch [*Menyailo et al.*, 2002b], possibly explaining the lower net N_2O production observed under aspen than under conifers.

3.4. Possible Limitations of ^{18}O Application

[20] We note four possible limitations of the method presented: (1) contributions of nitrifier denitrification; (2) limitation of denitrifying bacteria by available C, (3) dilution of the applied $^{18}O\text{-}NO_3^-$ by nitrification and (4) exchange of oxygen between intermediates produced in denitrification and water.

[21] Our approach does not account for nitrifier denitrification, a process which can contribute to N_2O production as revealed in pure culture studies [*Shaw et al.*, 2006]. However, knowledge about the contributions of nitrifier denitrification to N_2O efflux from soils is limited [*Wrage et al.*, 2001]. Use of our technique in combination with parallel incubations using $^{15}N\text{-}NO_3^-$ and $^{18}O\text{-}H_2O$ [*Wrage et al.*, 2005] could provide insight on the contributions of all three processes to total N_2O efflux.

[22] In the experiments we conducted, our goal was to assess the proportions of nitrifier- and denitrifier-derived N_2O in the absence of substrate limitations. Addition high amounts of NO_3NH_4 should relieve NO_3^- limitation of denitrifiers and NH_4^+ limitation of autotrophic nitrifiers. However, the shift in resource balance could induce C limitation of heterotrophic denitrifying bacteria, causing their contributions to total N_2O efflux to be underestimated. This limitation is not so easy to overcome since addition of C-source would most likely promote rapid growth of denitrifying bacteria making denitrification a dominant source of N_2O.

[23] The third possible limitation is easier to overcome by adding high amounts of NO_3^-. Nitrification, by forming new unlabeled NO_3^- and diluting the applied $^{18}O\text{-}NO_3^-$, was likely a minor source of error in the present study because we applied very high amounts of NO_3^- (250 mg kg^{-1}) compared to gross nitrification rates in these soils: 1–2 mg kg^{-1} d^{-1} [*Menyailo and Hungate*, 2006]. Avoiding dilution of applied $^{18}O\text{-}NO_3^-$ was the major reason for high N application. However, future studies at lower N application

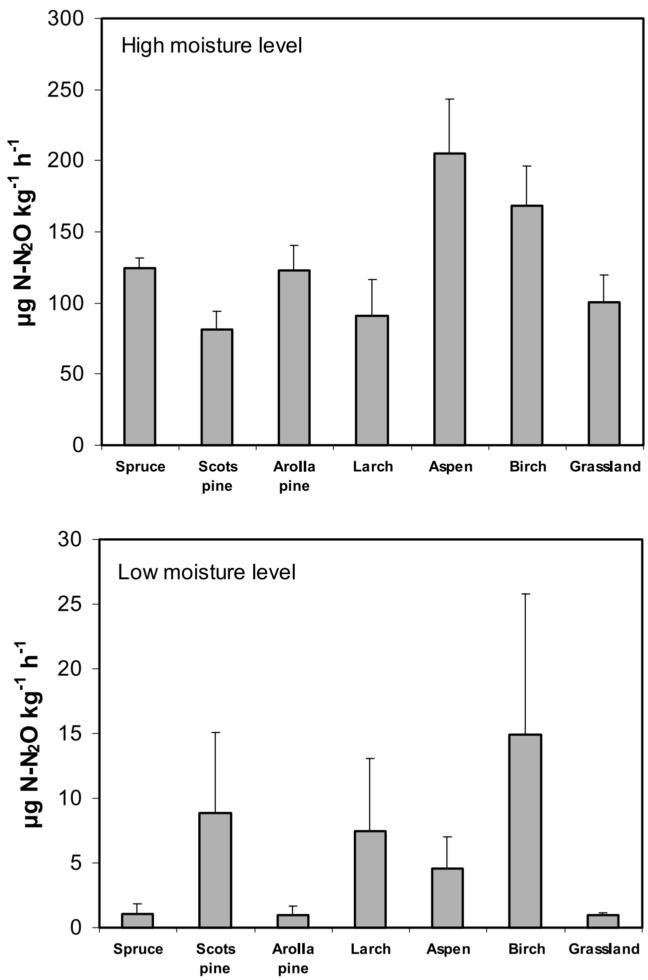

Figure 4. Rate of N_2O production at the same two moisture levels in soil samples under six tree species and grassland (n = 2). Soil moisture enhanced the efflux of N_2O by 10–100 times. When the flux is high, the largest amount of N_2O evolved from soils beneath hardwood tree species (aspen and birch).

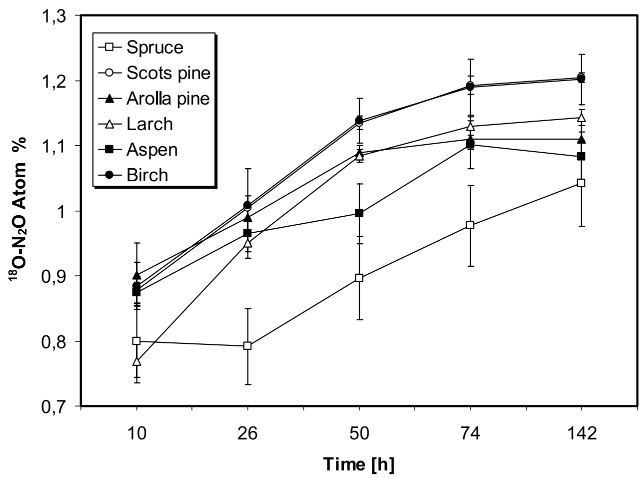

Figure 5. Oxygen isotopic enrichment (^{18}O) of N_2O evolved from the soil samples under different tree species at high soil moisture during 142-hour incubation (n = 2). The significant increase in ^{18}O-N_2O with incubation time indicates the increase in denitrifier-derived N_2O.

should also estimate the contribution of nitrification to ^{18}O-NO_3^- dilution. This can be easily achieved by measuring gross nitrification rate with isotope pool dilution technique.

[24] The fourth limitation, exchange of oxygen between water and denitrification intermediates (NO_2^- and NO), also deserves consideration. The exchange of water oxygen with nitrite and nitric oxide has been demonstrated for some denitrifying bacteria [*Garber and Hollocher*, 1982; *Ye et al.*, 1991; *Shearer and Kohl*, 1988], but the degree of exchange varies greatly among bacterial strains and may be related to biochemistry of nitrite reduction [*Ye et al.*, 1991]. Bacteria possessing the heme-type nitrite reductase, as *Pseudomonas chlororaphis* does [*Ye et al.*, 1993], were shown to catalyze relatively large amounts of exchange (39–76%) [*Ye et al.*, 1991], while *Pseudomonas aureofaciens*, known to possess the copper-type nitrite reductase [*Glockner et al.*, 1993] was shown to cause relatively little incorporation of oxygen atoms from water into N_2O (around 6%) [*Ye et al.*, 1991]. *Casciotti et al.* [2002] recently reported the low incorporation of oxygen isotopes of water into the N_2O by *Pseudomonas aureofaciens*: While in some cases up to 10% of oxygen in N_2O originated from water, in most cases incorporation was frequently less than 3%. It is thus possible that the relative abundance of denitrifying bacteria possessing either heme- or copper-type nitrite reductase will determine the applicability of ^{18}O isotopes for separation nitrification and denitrification. However, the ecological significance and relative abundance of the two groups of nitrite reductases is poorly understood.

[25] We have two lines of evidence that the exchange of oxygen between nitrogen oxides and water is minor in the

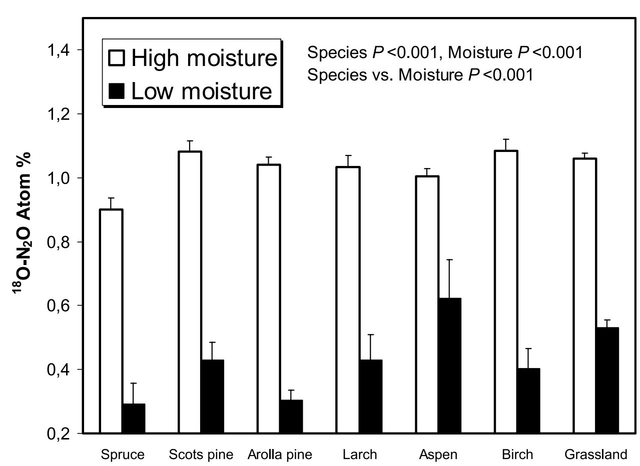

Figure 6. Oxygen isotopic enrichment (^{18}O) of N_2O evolved from the soil samples under different tree species at high and low moisture levels. The mean and standard errors for all sampling points are presented. High soil moisture increases the ^{18}O-N_2O values compared to low soil moisture, reflecting the higher contribution of denitrifying bacteria to N_2O efflux under the presumably more anaerobic conditions.

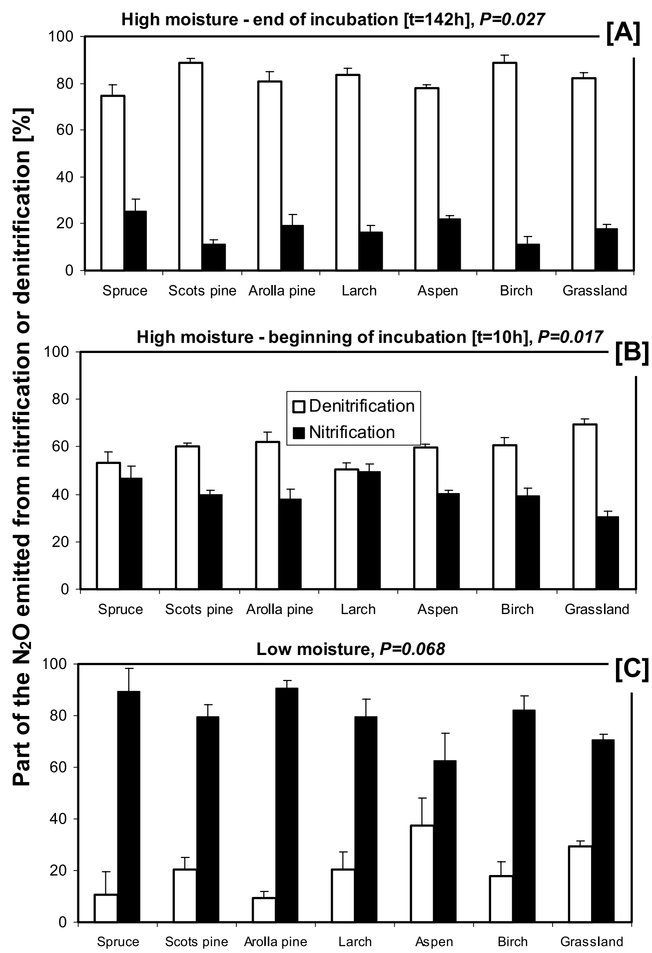

Figure 7. Estimated proportion of nitrifier- and denitrifier-derived N_2O for the high moisture treatment (a) at the beginning and (b) at the end of the incubation, and (c) for the low soil moisture treatment. The highest contribution of denitrification was at high soil moisture and at the final stage of the incubation. Nitrification contributes to N_2O efflux at low soil moisture, but when the rate of N_2O itself is very low (Figure 4).

studied soils. First, if large rates of exchange had occurred we would not have observed the progressive enrichment of ^{18}O-N_2O as our incubation proceeded (Figure 5). Rather, the ^{18}O-N_2O would have decreased or remained more or less constant. The enrichment in ^{18}O in concert with presumed increased O_2 deficiency, promoting denitrification, suggests that the exchange of oxygen between denitrification intermediates and water is minor. The second argument for defending our method is that in our recent study of N_2O isotope discrimination at natural abundance [*Menyailo and Hungate*, 2006] we demonstrated parallel enrichment of nitrogen and oxygen isotopes in N_2O under strictly denitrifying conditions (absence of oxygen and C_2H_2 presence), in the same soil samples we used in the present study. Both oxygen and nitrogen isotopes followed the Rayleigh distillation and were most depleted at the beginning of the incubation; as denitrification proceeded and NO_3^- became limiting, both ^{15}N-N_2O and ^{18}O-N_2O gradually became more enriched.

[26] These two arguments provide evidence that oxygen exchange was likely not a problem in these soils, probably owing to dominance of denitrifying species not actively exchanging oxygen of water with gaseous intermediates or, again, owing to high amounts of NO_3^- applied since O-isotopes exchange appears to be more important when NO_2^- or NO are final electron acceptors [*Ye et al.*, 1991]. However, future studies should address the questions of distribution and abundance of denitrifying bacteria possessing heme- and copper-type nitrite reductases (with varied exchange rates of oxygen) in different soils and ecosystems.

4. Conclusion

[27] Use of ^{18}O isotopes in NO_3^- provides additional insight in distinguishing the major biological sources of soil-derived N_2O, nitrification and denitrification. Our method was sensitive and allowed tracking a shift in denitrification and nitrification even within a relatively short incubation time (several days). Although our measurements were conducted under laboratory conditions, the principles ought to be applicable to studies targeting the separation of nitrifier- and denitrifier-derived N_2O in the field. Our results also suggest that tree species influence not only the rate of N_2O production but also the mechanisms of N_2O production. We demonstrated that the response to altered soil moisture of the processes responsible for N_2O production depends on the tree species. This has importance for predicting future N_2O fluxes from forest ecosystems with altered tree species composition and precipitation patterns.

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