Nitrous oxide consumption potentials of well-drained forest soils in southern Québec, Canada

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ABSTRACT

To establish the major controls on N_2O consumption by forest soils, we conducted laboratory incubations of sixteen samples from four soil types, two organic and two mineral, varying in overlying forest vegetation (sugar maple, American beech and eastern hemlock). The fastest potential consumption of N_2O occurred under anoxic conditions with little soil nitrate and under elevated headspace N_2O concentration. Potential N_2O consumption rates were fastest in organic soils under hemlock and beech trees (111 and 75 ng N_2O-N g^{-1} d^{-1}, respectively) compared to mineral soils under beech and maple trees (45 and 41 ng N_2O-N g^{-1} d^{-1}). Organic soils showed faster N_2O consumption rates than mineral soils, possibly due to larger organic C levels and higher C:N ratios. Acetylene treatment confirmed that denitrification was the process underlying N_2O consumption. These results suggest that soils regularly consume N_2O with varying magnitude, most likely in anoxic microsites throughout the soil profile and that the potential for N_2O consumption is larger in organic than in mineral forest soils.

INTRODUCTION

Soils emit nitrous oxide (N_2O), a greenhouse gas, into the atmosphere and account for 10 of the 16 Tg nitrogen (N) of the total N_2O released into the atmosphere each year (IPCC 2001; IPCC 2007). Approximately 4 Tg comes from agricultural soils, thus of anthropogenic origin, while the remaining 6 Tg are attributed to emissions from soils under natural ecosystems (IPCC 2001; IPCC 2007). Although forest soils are net sources of N_2O to the atmosphere, there is evidence that soils may also consume atmospheric N_2O (Arah *et al*. 1991).

The capacity of soils to act as sources or sinks of N_2O is the result of dynamic microbial processes of consumption and production occurring within the soil profile (Chapuis-Lardy *et al.* 2007). Denitrification and nitrification are the two dominant mechanisms of N_2O production; other biological and abiological processes (such as assimilatory and dissimilatory nitrate reduction and chemodenitrification) are thought to contribute < 1% of N_2O emissions (Chapuis-Lardy *et al.* 2007). The mechanisms of N_2O consumption in soils are less well studied and both atmospheric N_2O and locally produced N_2O can be taken up by soils and reduced to N_2 as the last step in the denitrification process, owing to the N_2O reductase enzyme (named Nos; Chapuis-Lardy *et al.* 2007). Nitrifiers have also been shown to play a role in N_2O consumption by reducing NO_2 to N_2, a process called nitrifier denitrification (Megonigal *et al.* 2004). Alternative processes of consumption have also been suggested, including aerobic denitrification and assimilatory reduction to ammonia (NH_3) (Chapuis-Lardy *et al.* 2007, Vieten *et al.* 2008).

There is a lack of knowledge of the potential for and the controllers of N_2O consumption in forest soils, though mechanisms have been identified. Few studies have investigated N_2O consumption directly, yet many focusing on emissions have nevertheless cited negative fluxes (from the atmosphere to the soil), which have often gone unexplained (Chapuis-Lardy *et al.* 2007). Additionally, even where net emission is observed, consumption processes can exert a significant effect on its magnitude (Arah *et al*. 1991). Much of the uncertainty leading to the large range in estimated forest soil emissions is related to the possibility of an underestimation of the potential for N_2O consumption, which could depress estimated emissions (Ullah *et al.* 2008).

In other work complementary to the present study, at two deciduous forest sites in southern Quebec, Mont St. Hilaire (MSH) and Morgan Arboretum (MA), we measured soil N_2O fluxes along hill slope catenas. While overall N_2O emission has been observed from the forest soils over the growing season, net consumption of N_2O was observed from well-drained soils at both sites during several summer sampling dates in 2006, particularly in June and July, though the rates remained small (Unpublished data). N_2O consumption rates ranged from 3.1 ± 1 to 6.0 ± 0.5 μg N_2O-N m^{-2} h^{-1} in well-drained soils under American beech (*Fagus grandifolia*) and sugar maple (*Acer saccharum*) at the MSH and MA sites, ranging from 0.1 to 22 μg N_2O-N m^{-2} h^{-1} in these soils. Overall, there was a net emission of N_2O to the atmosphere from well-drained soils under American beech and sugar maple, averaging 3.0 ± 0.7 and 5.4± 0.3 μg N_2O-N m^{-2} h^{-1}, respectively, when N_2O consumption rates were included in the calculation. When consumption rates were excluded from the calculation, net emissions rates averaged 5.5 ± 0.5 and 6.5 ± 0.6 μg N_2O-N m^{-2} h^{-1}, respectively (Unpublished data). Chapuis-Lardy *et al.* (2007) cited two studies in which N_2O consumption was observed in temperate deciduous forests, with rates ranging from 0.6 to 66 μg N_2O-N m^{-2} h^{-1} (Dong *et al*. 1998, Goossens *et al*. 2001). This suggests that N_2O consumption through denitrification is occurring within soils and can have significant impacts on net and average fluxes of N_2O from the soils.

The range of environmental factors influencing N_2O fluxes from these soils is broad, including both biogeochemical factors, such as NO_3, NH_4, and organic C availability, as well as physical factors such as soil texture, porosity, moisture, and temperature (Megonigal *et al*. 2004, Chapuis-Lardy *et al.* 2007, Ullah *et al*. 2008). These factors may affect the soil microbial populations, favoring certain functional groups and processes over others (Megonigal *et al*. 2004), as well as differences among topographic position and forest type (Ullah *et al*. 2008). Knowledge of the ways in which these factors control N_2O fluxes in different soils is incomplete, particularly as related to consumption processes (Chapuis-Lardy *et al*. 2007); in general, factors that limit N_2O diffusion appear to encourage consumption, including low mineral N levels and high moisture contents, suggesting denitrification as a principal mechanism for this consumption (Bandibas *et al.* 1994, Megonigal *et al*. 2004). In addition, the enzyme responsible for N_2O reduction in denitrifiers (Nos) is known to be particularly sensitive to

pH and oxygen (O_2) (Chapuis-Lardy *et al*. 2007).

We hypothesized that a) wet soil conditions and anaerobic processes impose N_2O consumption in these forest soils through denitrification; b) that soils with low available mineral N as substrate for N_2O production during denitrification under anoxic conditions may force full reduction and uptake of atmospheric N_2O; and c) that high organic C contents in the soil may increase microbially available energy stores and encourage atmospheric N_2O consumption, when soils undergo NO_3 limitation. To test these hypotheses, we measured potential N_2O consumption rates in sixteen soil samples collected from 4 soil types [two organic and two mineral] in the MSH and MA sites in laboratory incubations under elevated headspace N_2O concentrations to establish their potentials for N_2O consumption and to identify key conditions that favor this consumption.

MATERIALS AND METHODS

Study Sites

The two sites used in this study are representative of southern Québec mixed deciduous forest with a combination of tree species: American beech, sugar maple, yellow birch (*Betula alleghaniensis*), striped maple (*Acer pensylvanicum*), white ash (*Fraxinus americana*) and eastern hemlock (*Tsuga canadensis*). The Mont St. Hilaire site is located within an old growth forest occurring on one of the Monteregian hills, approximately 30 km east of Montreal. Two types of well-drained soils were sampled at this site: a sandy loam Brunisol underlying beech-dominated stands and a Podzol underlying hemlock-dominated stands, referred to as ‘beech’ and ‘hemlock’, respectively. The Morgan Arboretum site occurs in semi-managed forest located on the western tip of the island of Montreal. Sugar maple is the dominant tree species at this site, overlying well-drained, sandy loam soils referred to as ‘maple’.

Soil collection and preparation

Four plots (1 m^2 each) were randomly selected for each of the three soils. In November 2007, soil cores (10 cm diameter) were taken randomly from each of four plots to a depth of 10 cm and bulked, transferred to the laboratory and refrigerated until

further analysis. While the upper 10 cm of the maple (mineral) and hemlock (organic) soils showed no soil horizon change, the beech soils contained an organic horizon (O-horizon) overlying the mineral soil horizon (A-horizon). For these beech cores, the soil was separated into the organic (0 to 5 cm) and mineral (5 to 10 cm) horizons for separate analysis. The four resulting soils used in this study are: beech mineral, beech organic, hemlock organic and maple mineral. Each of the 16 soil samples (4 plots each for 4 soil types) was homogenized manually and sieved (< 2 mm), then analyzed for soil moisture and pH in water and extracted and analyzed for total dissolved N (TDN), dissolved organic C (DOC), NO_3 and NH_4 contents, as described in Ullah *et al*. (2008). Samples collected from 4 sampling points in each plot for N_2O consumption incubation and soil analysis incorporated the spatial variability within each plot.

Soil Leaching and Pre-incubations

Soil leaching and pre-incubations were performed in January 2008 on field moist soil samples collected from the four locations in the two watersheds to create conditions that favor N_2O consumption hypothesized above, including NO_3 limitation and anoxic conditions, before testing individual hypotheses under 8 treatments. To leach NO_3, 30 g of a soil at a time was leached by gravity in 60 ml syringes with the plungers removed and a Whatman GF/D filter placed at the tip. Three sequential washes of 15 ml de-ionized water (DI) were passed through the soils before returning them to field moisture conditions by applying pressure with the syringe plungers. Leachates were collected to ensure that soils were returned to field moisture conditions; these leachates were subsequently acidified and analyzed for DOC and TDN contents. After leaching, 15 g of soils was weighed into 150 ml serum bottles and 20 ml of DI was added. The bottles were capped tight, flushed with oxygen-free N_2 gas for 1 hour to induce anoxic conditions and incubated at room temperature for 5 days to further exhaust soil NO_3 through denitrification. Gas samples for N_2O concentration determination were collected from the headspace of the pre-incubated leached soil samples at 0, 24, 72 and 120 hours for one set of incubations, to test for the expected NO_3-depletion process occurring in the soils.

After leaching and pre-incubation, soils were incubated under 8 treatments, including 6 treatments applied to the leached and pre-incubated soil samples, 1 treatment

to unleached but pre-incubated samples and 1 treatment to unleached and not pre-incubated soil. A brief description and rationale of each treatment follows, with the procedures used:

1 Baseline

This treatment involved incubation of leached and pre-incubated soils under elevated headspace N_2O concentrations to investigate if NO_3 limitation and anoxic conditions would result in larger potential N_2O consumption rates. 15 g of leached and pre-incubated soil was weighed into a 150 ml serum bottle, followed by the addition of 20 ml of DI water. The bottles were capped with a gas-tight septa, flushed with oxygen-free N_2 gas for 1 hour to induce anoxic condition. After flushing, the headspace N_2O concentration in the bottles was raised to 2 ppm to ensure unlimited supply of N_2O and be able to quantify potential N_2O consumption rates. The incubation was performed on soil slurries on a rotary shaker at 75 rpm to encourage equilibrium solubility of the headspace gases. The incubation period lasted 24 hours, with 5 cm^3 gas samples taken at 0, 6, 12 and 24 hours, stored in pre-evacuated glass vials until analysis for N_2O concentration on a Shimadzu 14-A gas chromatograph equipped with a 6 m long porapack Q column and an electron capture detector. The end of the the GC column had a 4 valve electronic Valco Valve attached to it, which was timed to vent off separated O_2 coming out of column to avoid loss in the dector sensitivity for N_2O detection. Once the O_2 was vented off, the valve swiched back and directed the subsequently separated N_2O in the column into the detector. The column temperature was adjusted to 60 ^oC and that of the detector to 310 ^oC. Rate of N_2O consumption or emission was calculated from the change in concentration over the sampling duration using a linear equation obtained through a calibration curve of known N_2O standards.

2 Glucose-amended

This treatment repeats the baseline conditions, with an additional amendment of glucose (approx. 0.8 mg C/g dry soil) to identify the effects of increased C availability, delivered through a needle-fitted syringe to distribute the solution evenly throughout the soil slurry.

3 NO_3-amended

This treatment repeats the baseline conditions, with an additional amendment of KNO_3

192 solution (approx. 15 µg NO_3-N /g dry soil), delivered through a needle-fitted syringe.
193 This treatment is a test of whether N_2O consumption is reduced when inorganic N is
194 readily available.
195 *4 Unleached*
196 This treatment repeats the baseline conditions on soils, which were unleached, but pre-
197 incubated to isolate the effects of this method of N-limitation.
198 *5 No N_2O amendment*
199 This treatment repeats the baseline conditions, excluding amendment of bottle
200 headspaces with N_2O, with headspace composed entirely of N_2 gas.
201 *6 Field moisture, aerobic*
202 This treatment is designed to remove the gas diffusion limitation imposed in the baseline
203 incubation. This was done by incubating the soils at field moisture instead of under slurry
204 conditions and under aerobic headspace conditions (but still amended with N_2O). Soils in
205 this treatment were unleached and not pre-incubated.
206 *7 Unleached, field moisture, no N_2O amendment*
207 This treatment represents field conditions, with unleached soils incubated at field
208 moisture under aerobic and unamended conditions with ambient air headspace.
209 *8 Acetylene-amended*
210 This treatment repeats the baseline conditions but with the additional amendment of 10%
211 acetylene in the headspace to inhibit the reduction of N_2O to N_2 and block nitrification,
212 thereby isolating the role of denitrification and confirming that N_2O consumption
213 occurred through denitrification and not nitrification.
214
215 **Statistical analysis**
216 The gas flux from each bottle was calculated as the slope of the linear regression
217 line best fitting the sample points over 24 hours. Average consumption or emission rates
218 and standard errors were calculated based on the four replicates for each soil type and
219 treatment. A small constant (the value of the largest emission) was added to each one to
220 render all values positive; values were then log-transformed to meet the assumption of
221 normality. These values were compared by ANOVA and Fisher LSD using Statistica 6,
222 both for differences in fluxes within soil type, by treatment, and among soil types, for

each treatment. Pearson correlation coefficients were calculated in SAS 9.1.

RESULTS

The hemlock organic soil sample had the highest DOC content (321 µg C g^{-1} dry soil), followed by beech organic (180 µg C g^{-1} dry soil), with beech and maple mineral samples having the lowest DOC content (49 and 41 µg C g^{-1} dry soil, respectively; Table 1). The same trend is observed for TDN, with the hemlock organic soil having the highest content (17.8 µg N g^{-1} dry soil) and maple the lowest (5.7 µg N g^{-1} dry soil). In terms of NO_3 and NH_4 contents, however, the hemlock organic soil had the lowest values (0.9 and 3.1 µg N g^{-1} dry soil, respectively), with beech mineral (3.7 and 5.9 µg N g^{-1} dry soil) and organic (3.4 and 5.6 µg N g^{-1} dry soil) exhibiting the highest contents of both ions, trailed by maple mineral, which showed slightly lower contents (2.7 and 5.0 µg N g^{-1} dry soil). Leaching slightly reduced DOC and TDN contents for all soils, though the hemlock organic soil showed a large reduction in DOC content (80 µg C g^{-1} dry soil) with leaching (Table 1).

N_2O exchange from pre-incubated soils was low, ranging from a net emission of 0.22 ng N_2O-N g^{-1} d^{-1} from the hemlock soil to a net consumption of 1.8 ng N_2O-N g^{-1} d^{-1} from the beech mineral soil. Pre-incubated leached soils showed significantly smaller N_2O fluxes than those incubated under elevated headspace N_2O (Fig. 1). Substantial net N_2O consumption was observed in the soils under the conditions hypothesized to facilitate N_2O reduction: treatment 1 with anoxic conditions and with 2 ppm N_2O-amended headspace (Fig. 1). The greatest potential for N_2O consumption was found in the hemlock organic soil, with a consumption of 111 ng N_2O-N g^{-1} d^{-1} (Fig. 1c), significantly larger ($p < 0.05$) than the beech organic soil with 74.5 ng N_2O-N g^{-1} d^{-1} (Fig. 1 b). The N_2O consumption rate was significantly slower in the two mineral soils: beech (45.2 ng N_2O-N g^{-1} d^{-1}) and maple (40.7 ng N_2O-N g^{-1} d^{-1}, Fig. 1a, d).

When soils were incubated at field moisture or under aerobic conditions, the N_2O consumption rate decreased greatly in all soils, even in the presence of high concentrations of N_2O in the headspace (treatments 6 and 7, Fig. 1). When the headspace remained unamended with N_2O, most soils switched to slow rates of N_2O emission, despite saturated and anoxic conditions (Treatment 5). For the incubation at field

moisture, under aerobic, unamended conditions, the average fluxes were near-zero for all soils (Incubation 7). Leaching with H_2O appeared to slightly increase the potential for N_2O consumption for most soils, but the average flux was significantly different from that of the unleached soils only for the beech mineral soil (Treatment 4, Fig. 1a).

The addition of organic C in the form of glucose (treatment 2) yielded a slightly faster N_2O consumption rate in the two beech soils, but was statistically indistinguishable from the baseline treatment 1 for all soils (Fig. 1). The addition of NO_3 solution to the soils clearly decreased the N_2O consumption rate for all soils, though not significantly (treatment 3). The addition of headspace acetylene (treatment 8) resulted in near-zero N_2O fluxes in all soils, canceling the strong consumption observed in treatment 1 and was statistically significant ($p < 0.05$) in all cases.

There were few significant differences in N_2O fluxes among the four soils for each treatment: significant ($p < 0.05$) differences occurred only in treatments 1, 2 and 4, with the hemlock and beech organic soils generally with the largest potential consumption rates (Fig. 1).

A correlation analysis revealed that the potential N_2O consumption rates under treatment 1 are significantly and negatively correlated with both DOC and original soil moisture, suggesting that soils with higher C contents and field moisture levels have a greater capacity to consume N_2O (Table 2). In addition, TDN showed a similar negative correlation with N_2O fluxes, though only significant at the 10% level.

DISCUSSION

The strong potential N_2O consumption rates obtained from the baseline incubation reveal that these well-drained forest soils have a significant capacity for N_2O reduction under conditions of anoxia and N limitation. Though conditions in this laboratory study are incomparable in many ways to field conditions, the occurrence of potential N_2O consumption rates at such magnitude suggests that these processes could play a significant role in determining net fluxes of N_2O from the soils, even though these soils are weak net sources of N_2O throughout most of the growing season. This result suggest a need for *in situ* N_2O consumption studies and the inclusion of N_2O reduction processes in the consideration of N cycling and gas fluxes in these soils, and forest soils in general.

The conditions which facilitated N_2O reduction in this study included: a) the imposition of enhanced N_2O consumption, through anaerobiosis and soil water saturation; b) low availability of electron acceptors, notably NO_3, achieved by leaching and pre-incubating soils; and c) high organic C contents to encourage reduction of N_2O as an alternate electron acceptor. The baseline incubation (treatment 1) showed that the combination of these factors yielded significant consumption in all four soils, both mineral and organic. The near-zero N_2O fluxes obtained under conditions similar to those in the field (treatment 7) represented a clear contrast to the baseline incubation. The remaining six treatments served to identify the effect of the different variables on potential N_2O consumption.

The glucose addition (treatment 2) increased N_2O consumption levels only (and then not statistically significant) in the beech soils, both organic and mineral. Soil C:N ratios (Table 1) influenced N_2O consumption, where beech and hemlock showed faster consumption rates and their C:N ratios were larger than those of soils under sugar maple. Cavigelli and Robertson (2001) also noted that the Nos enzyme is particularly sensitive to a low C:N ratio and stated that the organic C level in a soil is an important factor for soil denitrifier populations. This might explain the low baseline N_2O consumption of the maple mineral soil, as well as the absence of an increase in consumption upon glucose amendment. The hemlock organic soil may not have shown increased N_2O consumption upon glucose addition, owing to the high DOC levels of organic C in these soils. Larger nitrification rates in soils under the sugar maple compared to those under American beech and hemlock in these plots may have led to the evolution of denitrifiers with low affinity for N_2O consumption in soils under sugar maple trees (Ullah and Moore, 2009). Additionally, *in situ* N_2O consumption rates in soils under American beech were 2 times larger than under sugar maple in these sites (Ullah and Moore, in prep). We hypothesize that soils with larger soil C content and C:N ratio in deciduous forests consume more N_2O than soils under smaller C:N ratios. We suggest further microbial studies to validate this hypothesis under field conditions.

Our strong correlation between N_2O consumption and extractable DOC concentration, within the limited range of soils, is consistent with reports on the importance of organic C (as well as moisture, oxygen, disturbance and pH levels) in

316 determining N_2O consumption potentials, which may also reflect differences in microbial
317 communities among soil types (Parkin 1987, Cavigelli and Robertson 2001, Wallenstein
318 *et al.* 2006). Given the limited, non-significant increases in N_2O consumption upon
319 glucose amendment in the beech soils, further experiments are needed to determine
320 whether different and larger organic C amendments would have a significant effect, or
321 whether the microbial populations present in the soils limit the response over the 24-hour
322 incubation period.
323 The addition of NO_3 (treatment 3) and the incubation of unleached soils
324 (treatment 4) were performed to contrast N_2O consumption with those of the baseline
325 incubation (treatment 1), where an effort was made to eliminate as much NO_3 from the
326 soils as possible. This was to test whether denitrifiers will turn to an alternate, though less
327 energetically favorable electron acceptor, the N_2O provided through headspace
328 amendment (Bandibas *et al.* 1994), a technique of limiting the availability of electron
329 acceptors successfully employed in other studies (e.g. Firestone *et al.* 1980, Holtan-
330 Hartwig *et al*. 2000). This hypothesis was supported by our results, showing a decreasing
331 trend, although not statistically significant at $p < 0.05$, in treatments 3 and 4 (Figure 1).
332 The near-zero N_2O fluxes in pre-incubation conditions support the hypothesis that this
333 treatment lowered NO_3 in the soil, allowing the uptake and reduction of N_2O as an
334 alternate electron acceptor in the following treatments. Leaching the soils clearly
335 depressed total N levels in all cases (Table 1). The differences in N_2O consumption
336 between the leached, pre-incubated soils and the same soils with NO_3 added, reflect the
337 control of NO_3-availability: adding NO_3 after leaching and pre-incubating the soils
338 effectively cancels out the effect of the initial leaching. There was a decrease in N_2O
339 consumption by about one third upon NO_3 addition in the beech soils, whereas the effect
340 was less pronounced in the hemlock and maple soils.
341 Thus, while NO_3 availability is significant, other conditions such as anaerobiosis,
342 moisture and N_2O play key roles in determining whether N_2O consumption will occur.
343 The correlation between low NO_3 levels and N_2O consumption is widely cited, but most
344 studies go on to describe the conditions of anaerobiosis and saturation as predominantly
345 important (Blackmer and Bremner 1976, Firestone *et al.* 1980, Bandibas *et al.* 1994,
346 Holtan-Hartwig *et al.* 2000, Rosenkranz *et al.* 2006, Wallenstein *et al.* 2006). A possible

reason why a major decrease in N_2O consumption was not observed in treatments 3 and 4 is that the NO_3 was quickly exhausted, leading to a NO_3-limitation similar to that of the baseline and creating larger N_2O consumption later during the 24-hour incubation. Firestone *et al.* (1980) suggested that Nos enzyme could be sequentially produced during the incubation, resulting first in an increase and then a decrease in headspace N_2O concentration. We recommend further studies with ^{15}N tracers to validate this hypothesis.

Excess N_2O appears to be critical in its consumption as an alternate electron receptor upon NO_3-limitation (Mei *et al.* 2004). This is reflected in treatment 5, without the N_2O headspace amendment, where, for all but the hemlock soil, there was a small N_2O production (Fig. 1) indicating that, all other conditions being equal, when N_2O is not abundantly available in the soil pore spaces, N_2O consumption will likely be small, or is severely limited. The leaching and pre-incubation is effective at reducing available NO_3 as a substrate for denitrification but the small rates of N_2O indicate that complete reduction to N_2 (and thus N_2O consumption) may be limited due to lower soil pore space N_2O concentrations. Indeed, the lack of substrates for denitrifiers under treatment 5 likely limited their activity in either the production or consumption, resulting in the fluxes. For the hemlock soil, the large standard error makes the interpretation of the small consumption rates for this incubation ambiguous, but perhaps suggests that complete reduction can occur under these circumstances, if enough organic C is available to encourage microbial activity, but this is variable and ephemeral. Fast denitrification rates are often associated with high organic C in soils (Parkin 1987, Wrage *et al.* 2001) and more work could be done to establish the effect of varying the headspace N_2O concentrations, from 2 ppm (treatment 1) to ambient.

In treatment 6, soils were incubated aerobically and at field moisture to identify the effect of anoxic conditions, resulting in a significant decrease in N_2O consumption. Anoxic conditions play a critical role in N_2O consumption in soils, for which there is support in the literature:

a) When oxygen is allowed to diffuse into the soil, denitrifier activity and Nos activity in particular are impeded and restricted to microsites of anoxia (Firestone *et al.* 1979, Bandibas *et al.* 1994, Cavigelli and Robertson 2001).

b) Low moisture allows greater gas diffusion into and out of the soil profile and any

remaining NO_3 reduced to N_2O is able to diffuse out of the soil without further reduction to N_2, whereas higher moisture content facilitates N_2O entrapment and reduction to N_2 (Clough *et al.* 2005; Ullah et al. 2005).

c) N_2O present in the headspace diffuses into the soil profile more easily under low moisture conditions, which paradoxically may tend to encourage consumption in the drier soils by increasing the availability of the N_2O as a substrate in the redox chain (Bandibas *et al.* 2004). This could be the reason why N_2O fluxes remained negative, though reduced in magnitude, in treatment 6. This effect is evidenced further in treatment 7, where in addition to the removal of the gas diffusion limitation, the N_2O headspace amendment is removed (as well as leaching), and fluxes diminish to near-zero or slight production. Clough *et al.* (2005) suggest that this is due to the decreased time in which potential reduction can occur under low moisture conditions because of increased gas diffusion and prevalence of oxic conditions in soil profile.

d) Though the presence of anoxic conditions appears to exert a stronger control over N_2O consumption than NO_3 availability, the NO_3-limitation is likely required initially to encourage N_2O consumption as the predominant denitrifier activity in anoxic, saturated conditions, as suggested in treatments 3 and 4. The two controls are interdependent, and their importance can both be traced to the availability of the various reactants in the denitrification redox chain, which in all cases is concentration and diffusion-dependent.

The addition of acetylene in treatment 8 inhibited the reduction of N_2O to N_2 and removed the strong N_2O consumption under treatment 1 (Schuster and Conrad 1992). This eliminates the possibility that other factors, such as simple diffusion into soil water, is causing the decrease in concentration of N_2O in the headspace over the incubation period, confirming that the N_2O is in fact being reduced to N_2 through denitrification. Denitrification is clearly implicated as the process by which this reduction occurs under baseline conditions since the acetylene amendment also inhibits nitrification processes. N_2O is not accumulating in the headspace, implying that the N_2O being reduced in treatment 1 was primarily amended headspace N_2O through denitrification.

These well-drained soils are capable of consuming N_2O, so the summer field consumption that first motivated this study are likely not anomalous. Although great care needs to be taken in extrapolating these results to field conditions, the potential for N_2O

consumption in the field likely results from denitrification processes occurring in isolated anoxic, wet microsites within the soil, where locally produced N_2O can be retained and reduced to N_2. This N_2O consumption is usually not detectable in the field as emission rates are slow emissions, though the emission rate may be decreased by N_2O consumption (Ullah *et al*. in prep.). Net field N_2O consumption could be anticipated after heavy rainfall, where soils are strongly NO_3-limited and with large organic C contents (Seitzinger *et al.* 2006).

CONCLUSIONS

Well-drained forest soils in southern Québec exhibited potential N_2O consumption through denitrification when incubated under anoxic and saturated moisture conditions, and when amended with high levels of atmospheric N_2O. Mineral N limitation within the soils likely stimulated the reduction of N_2O to N_2. Organic soils showed generally greater N_2O consumption potentials than mineral soils. Conditions favoring N_2O consumption may occur in wet, anaerobic microsites within the soil profile, and such consumption processes could bear significantly on the net flux of N_2O from these soils. Our results also suggest that soil with larger soil C:N ratios exhibiting lower nitrification rates may possess higher affinity for N_2O consumption than soils with smaller ratios.

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516 **TABLES**

517 Table 1. Chemical properties of the 4 soil types and leaf litter input and litter C:N ratio.

Variables	Beech mineral	Beech organic	Hemlock organic	Maple mineral
pH	4.7 ± 0.5	4.7 ± 0.5	4.5 ± 0.39	5.5 ± 0.03
Bulk density (g cm^{-3})	0.99 ± 0.21	0.56 ± 0.10	0.48 ± 0.1	1.19 ± 0.04
DOC (μg C g^{-1} dry soil)	49 ± 2	180 ± 18	321 ± 29	41 ± 2
DOC leached (μg C g^{-1} dry soil)	45 ± 2	150 ± 14	241 ± 18	36 ± 2
TDN (μg N g^{-1} dry soil)	8.5 ± 2.4	15.6 ± 2.3	17.7 ± 0.9	5.7 ± 2.3
TDN leached (μg N g^{-1} dry soil)	8 ± 2.3	12.1 ± 1.1	14.4 ± 0.7	4.8 ± 1.3
NO_3 (μg N g^{-1} dry soil)	3.7	3.4	0.9	2.7
NH_4 (μg N g^{-1} dry soil)	5.9	5.6	3.1	5
Soil C:N ratio (0-10 cm depth)	26 ± 1.3*		25 ± 1.5	16 ± 0.7
Leaf litter N input (g m^{-2})	4.6 ± 1.4**		1.9 ± 0.3	4.9 ± 0.1
Leaf litter fall C:N ratio	61 ± 6**		71 ± 3	52 ± 0

518

519 * C:N ratio in soils under beech trees represent an average of both organic and mineral layer as only a 0-10 cm depth sample was
520 taken for this purpose. ** Represents total litter N input on the soil surface and its C:N ratio.

521 Table 2. Pearson correlation coefficient (*r*) between the properties of the four soils (see
522 Table 1) and the N_2O consumption rate under treatment 1. Coefficients with *p*-values that
523 are significant at the 5% level are listed in **bold** text and those at the 10% level are
524 *italicized*.
525

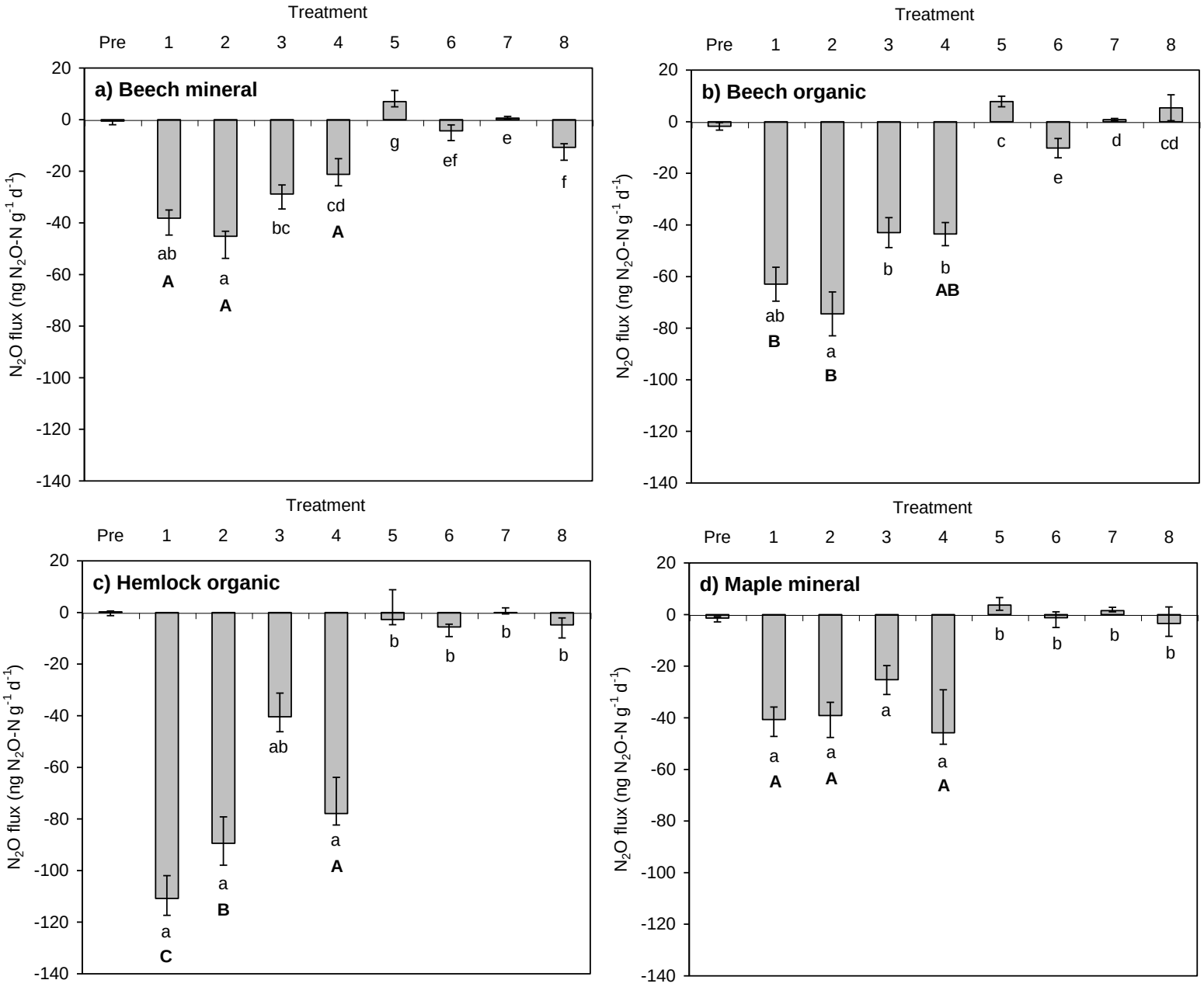
	N_2O flux	DOC	TDN	NO_3	NH_4	pH	Bulk density
N_2O flux	-						
DOC	**-1.00**	-					
TDN	*-0.92*	*0.94*	-				
NO_3	0.82	-0.78	-0.52	-			
NH_4	0.83	-0.80	-0.55	**1.00**	-		
pH	0.63	-0.67	-0.85	0.10	0.13	-	
Bulk density	0.71	-0.73	-0.83	0.30	0.34	*0.94*	-
Field soil moisture	**-0.97**	**0.98**	**0.97**	-0.68	-0.70	-0.79	-0.85

526

527

FIGURES

Figure 1. Average N_2O exchange rates (± standard error) for the four soil samples under the Pre-incubation and the 8 treatments: 1 Baseline; 2 Glucose-amended; 3 NO_3-amended; 4 Unleached; 5 No N_2O amendment; 6 Field moisture, aerobic; 7 Unleached, field moisture, no N_2O amendment; 8 Acetylene-amended. Negative values indicate consumption by the soil. Statistically significant differences ($p < 0.05$) among treatments for each soil for are indicated by lower case lettering and among the four soils under treatments 1, 2, 3 and 4 are indicated by bold upper case lettering.


a) Beech mineral
b) Beech organic
c) Hemlock organic
d) Maple mineral
Treatment
Pre 1 2 3 4 5 6 7 8
N2O flux (ng N2O-N g-1 d-1)
20 0 -20 -40 -60 -80 -100 -120 -140
ab A
a A
bc
cd A
g
ef
e
f
ab B
a B
b
b AB
c
e
d
cd
a C
a B
ab
a A
b
b
b
b
a A
a A
a
a A
b
b
b
b

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