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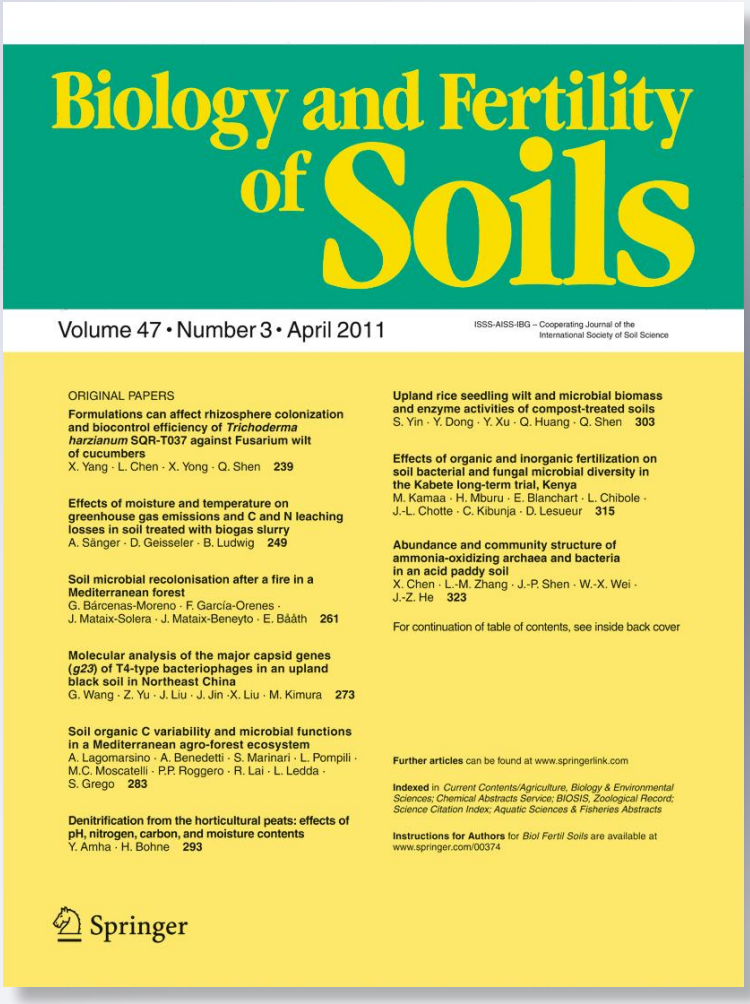

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ORIGINAL PAPER

Denitrification from the horticultural peats: effects of pH, nitrogen, carbon, and moisture contents

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Abstract Denitrification plays an important role in N-cycling. However, information on the rates of denitrification from horticultural growing media is rare in literature. In this study, the effects of pH, N, C, and moisture contents on denitrification were investigated using four moderately decomposed peat types (*oligotrophic, mesotrophic, eutrophic*, and *transitional*). Basal and potential denitrification rates (20°C, 18 h) from the unlimed peat samples varied widely from 2.0 to 21.8 and from 118.9 to 306.6 μg (N_2O+N_2)–N L^{-1} dry peat h^{-1}, respectively, with the highest rates from the eutrophic peat and the lowest from the transitional one. Both basal and potential denitrification rates were substantially increased by 3.6–14- and 1.4–2.3-fold, respectively, when the initial pH (4.3–4.8) was raised to 5.9–6.5 units. Emissions of (N_2O+N_2)–N from oligotrophic, mesotrophic, and transitional peats were markedly increased by the addition of 0.15 g NO_3–N L^{-1} dry peat but further additions had no effect. Denitrification rates were increased by increasing glucose concentration suggesting that the activity of denitrifiers in all peat types was limited by the low availability of easily decomposable C source. Increasing moisture contents of all peats from 40 to 50% water-filled pore space (WFPS) did not significantly ($p>0.05$) increase (N_2O+N_2)–N emissions. However, a positive effect was observed when the moisture contents were increased from 60% to 70% WFPS in the eutrophic peat, from 70% to 80% in the transitional, from 80% to 90% in the oligotrophic and from 70% to 90% in the mesotrophic peat. It can be concluded that liming, N-fertilization, availability of easily decomposable C, and moist condition above 60% WFPS could encourage denitrification from peats although the rates are greatly influenced by the peat-forming environments (eutrophic>mesotrophic>oligotrophic>transitional types).

Keywords Basal and potential denitrification · Glucose · Liming · Nitrate · Peat-forming environment · Water-filled pore space

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Introduction

Denitrification is a four-reaction process through which nitrate (NO_3^-) is converted to nitrite (NO_2), nitric oxide (NO), nitrous oxide (N_2O), and molecular nitrogen (N_2) by facultative anaerobic microorganisms that use organic compounds as electron donor and nitrogen oxides (ion and gaseous form) as terminal electron acceptor (Davidson et al. 1993; Vymazal 2007). Apart from the availability of NO_3^-–N (Matson and Vitousek 1990) and readily oxidizable organic substances (Duxbury et al. 1982), the production and emission of N_2O/N_2 via denitrification can be influenced by several soil and environmental factors including the water content (Klemedtsson et al. 1991), pH (Brady and Weil 1999), temperature (Saad and Conrad 1993), oxygen availability, and the redox potential (Davidson et al. 1993; Vymazal 2007).

Measuring denitrification from soils has paramount importance, as the gas produced (N_2O) is a potent greenhouse gas that contributes to global warming (Drury et al. 1991). Moreover, the loss of inorganic N from root zone via gaseous emission reduces both crop productivity and the efficacy of expensive N fertilizers. Knowledge of soil denitrification is, therefore, essential for determining soil N budget, as the process plays a central role in the N

cycle (Tiedje et al. 1989). Consequently, denitrification has been a subject of thorough investigation in the agricultural and forest soils. Denitrification N losses from agricultural soils were reviewed by Nieder et al. (1989) and ranged from 0–200 kg Nha^{-1} $year^{-1}$ with the highest emissions from vegetable production system (Ryden and Lund 1980). Although N-losses from horticultural production system are expected to be significant, experimental works on this topic are scarce.

Horticultural crops in greenhouse and nursery are grown mostly on peat medium as it retains large volumes of water, air, and plant nutrients in readily available forms (Robertson 1993). However, the physical and chemical properties of peats used in horticulture vary considerably (Amha et al. 2010) depending on their degree of decomposition and botanical composition (Bohlin et al. 1989), and the peat-forming environments (Shotyk 1988; Steinmann and Shotyk 1997). We, therefore, hypothesized that (1) the rate of denitrification from the horticultural peats is influenced by the respective peat-forming environments (*eutrophic*, *oligotrophic*, *mesotrophic*, and *transitional* types) and (2) the rate of denitrification from a given horticultural peat is influenced by the availability of easily decomposable C source to heterotrophic microorganisms and our managements in nursery (i.e., NO_3^-–N-fertilization, irrigation, liming).

The objectives of this study were: (1) to determine the basal and potential denitrification from limed and unlimed peat samples under anaerobic condition and (2) to evaluate the effect of NO_3^-–N, glucose-C, and moisture contents on the production of (N_2O+N_2)–N. Basal denitrification rate is predominantly controlled by the availability of the intrinsic organic C and NO_3^- contents (Drury et al. 1991) and was conducted to obtain valuable information about the activity of indigenous denitrifiers in the tested peat samples. In contrast, the potential denitrification rates estimate the amount of denitrified-N from peats when both easily decomposable organic C and NO_3^-–N are not limiting (Pell et al. 1996). The NO_3^-–N and moisture experiments were designed to represent wide ranges of fertilization and irrigation used for the production of horticultural crops, respectively. Glucose was used to stimulate microbial activity.

Materials and methods

Peat samples

The rates of denitrified-N were measured from four horticultural peats varying in their peat-forming environments (*eutrophic*, *oligotrophic*, *mesotrophic*, and *transitional* peat types). Classification of peats on the basis of peat-forming environment has been reported by Stewart and Kantrud (1971). The oligotrophic peat was evolved from ombrotrophic bog (Pütte 27, Estonia) that entirely depended on precipitation for its nutrient source; whereas, the mesotrophic and eutrophic peats were developed from fens (Vechta, Germany and Kikilla, Finland, respectively) that received minerals from precipitation, ground, and surface water. The transitional peat was obtained from the intermediate mire (mesotrophic–ombrotrophic interval, Osnabrück, Germany) where neither the precipitation nor surface and ground water dominates the nutrient balance. The botanical composition of each peat sample was determined by identifying the predominant peat-forming plant genus according to Heikurainen and Huikari (1952). All peats contained remnants of *Sphagnum*, *Carex*, *Bryales*, secondary particles, shrub, and woody plants. They were classified as moderately decomposed peats (H4–H5) by the von Post-humification scale (von Post 1924), where H1 represented a completely undecomposed peat and H10 as a completely decomposed peat.

Physicochemical analyses

Each peat type was delivered to us in three separate plastic bags and each bag was considered as a replicate. Peats were sieved (<5 mm) and analyzed for their physicochemical properties. Fresh bulk density (Vw_f; g L^{-1}) was determined according to the procedures outlined by VDLUFA (2002). Briefly, Vw_f was determined from the fresh weight of the subsample and the corresponding volume after compaction (i.e., by releasing a sample containing cylinder ten times from the height of 10 cm). The dry bulk density (D_{BD}; g L^{-1}), total pore space (P_S; in%), and particle density (P_D; g L^{-1}) were computed using the following formula:

$$D_{BD} = Vw_f * DM \quad (1)$$

$$P_S = \left(1 - \frac{D_{BD}}{P_D}\right) * 100 \quad (2)$$

$$P_D = \frac{100}{[(W_{om} / 1.55) + (W_{ash} / 2.65)]} \quad (3)$$

where, DM, W_{om}, and W_{ash} represent dry matter percentage (105°C, 48 h), organic matter, and ash contents, respectively. The W_{ash} and W_{om} contents, expressed in relation to the initial oven dry weight, were determined by dry combustion at 550°C for 16 h. The water content–water tension relationship was determined according to DIN EN 13041 (2000), and the air volume was calculated

as the difference of P_S and water volume at 1 kPa. Fresh sample was shaken with distilled water (1:5 *v*/*v*; 1 h at 40 rpm) and pH was measured from the suspension (pH-meter 761, Knick, Germany) and electrical conductivity from the filtrate (Tertacon® WTW, Weilheim, Germany). The initial NH_4^+–N and NO_3^-–N contents were determined using an autoanalyzer (Alpkem Corp., Origen, USA) after shaking fresh sample in 0.01 M $CaCl_2$ (1:4 *w*/*v*) for 1 h. Total C and total N were determined by an elemental analyzer (Vario MAX CN analyzer, Hanau, Germany) after calibrating the instrument with glutamine (Sigma-Aldrich Chemie GmbH, Germany) and other standard reference materials.

Samples preparation for incubation

The bulk sample in each bag was homogenized before to be used in subsequent incubation experimentations. Some subsamples were taken and adjusted for pH by adding 1.25 g $CaCO_3$ L^{-1} dry peat (99.99%; Merck, Darmstadt, Germany). The targeted pH was between 5.5 and 6.5, as most horticultural crops grown at this range (Reinikainen 1997). The moisture content of each peat type was adjusted to 30% of the respective water-filled pore space (WFPS; Eq. 4) by adding distilled and deionized water (if necessary).

$$\mathrm{WFPS} = \frac{W_m * D_{BD}}{\rho H_2O * P_S} * 100 \quad (4)$$

where, WFPS, W_m, D_{BD}, ρH_2O, and P_S represent water-filled pore space (%), gravimetric water content (mg/mg), peat dry bulk density (mg m^{-3}), density of water (mg m^{-3}), and total pore space, respectively.

The homogenized subsamples were monitored for their gaseous emissions in subsequent incubation experiments. Subsamples without liming (n=3) were also included for determining basal and potential denitrification rates from the bulk samples.

Basal denitrification

Basal denitrification rates were determined from limed and unlimed subsamples. Based on the predetermined dry bulk densities, triplicate subsamples (100 mL each) were packed inside the incubation glass jars (250 mL capacity each) and their respective moisture contents brought to 1 kPa (DIN EN 13041 2000) with distilled and deionized water. The headspace gas inside a closed glass jar was evacuated and filled with helium (He). Then, 10% of the headspace gas was replaced by acetylene so as to block the conversion of N_2O to N_2 (Yoshinari et al. 1977). Headspace sample was taken at initial and after 18 h of incubation period (20°C) and analyzed for N_2O using a Perkin–Elmer Autosystem XL gas chromatograph equipped with ^{63}Ni-electron capture detector.

Potential denitrification

The homogenized subsamples (n=3; 100 mL each) were amended with KNO_3 (0.4 g N L^{-1} dry peat) and glucose (0.8 g C L^{-1} dry peat). They were added inside the jars, and their respective moisture contents adjusted to 0 kPa (DIN EN 13041 2000) to create peat slurries. Similarly, unlimed bulk samples (n=3) were treated with 0.4 g NO_3^-–N and 0.8 g glucose-C L^{-1}, and the respective moisture contents were adjusted to 0 kPa. Both limed and unlimed subsamples were then incubated in the same way as of the basal denitrification experiment.

Nitrate experiment

This experiment was designed to measure the effect of NO_3^-–N availability on the emission of (N_2O+N_2)–N. Limed subsamples (n=3; 150 mL each) were taken and treated with KNO_3 solution at rate of 0, 0.15, 0.25, 0.4, 0.6, 0.8, or 1.0 g N L^{-1} dry peat. To stimulate the activity of microorganisms, glucose was added to each treatment at rate of 0.4 g C L^{-1} dry peat. Both fertilizer and glucose were mixed thoroughly with the subsamples and packed into a plastic pot (according to the respective bulk density in Table 1). Distilled and deionized water was added to the sample to achieve a final moisture content of 60% WFPS. Each container was then kept inside a 1.5-L glass jar, and the respective headspace gas (10% *v*/*v*) was replaced with acetylene. The headspace gas was analyzed for N_2O at the beginning and after 1, 2, 3, 4, and 5 days of incubation period. Once the headspace gas is removed (10 mL/sampling), sufficient acetylene was injected back into the closed jar to return the jar to its original pressure. The incubation temperature was 25°C.

Glucose experiment

The effect of organic C on the (N_2O+N_2)–N production was studied using seven glucose concentrations (0, 0.05, 0.1, 0.2, 0.4, 0.6, and 0.8 g C L^{-1} dry peat). In each treatment, KNO_3 solution was added at rate of 0.4 g N L^{-1} dry peat. The used C rates are realistic as the concentration of dissolved organic C (DOC) in the rhizosphere soil reaches up to 750 μg g^{-1} (Bremer and Kuikmann 1994). When computed in volume basis (assuming the mean soil dry bulk density of 1.0 kg L^{-1}), the corresponding DOC could be comparable to what we have added here. The amount of incubated materials, number of replications, final moisture content in the subsample, acetylene concentration in the headspace, incubation time, headspace sample size,

Table 1 Physical and chemical properties (mean ± standard deviation; $n=3$) and botanical composition of the tested peat types

Parameters	Classification based on the peat-forming environment[a]			
	Oligotrophic	Mesotrophic	Transitional	Eutrophic
Botanical composition				
Sphagnum (%)	50	35	60	40
Carex (%)	15	15	20	30
Bryales (%)	15	10	5	10
Clumps (%)[b]	10	30	10	10
Mixture (%)[c]	10	10	5	10
Physicochemical properties				
Decomposition degree[d]	H5	H5	H4	H5
Dry bulk density (g L^{-1})	89±0.7 b	109±1.3 a	80±1.8 c	76±2.6 c
Total pore space (% *v*/*v*)	93.7±1.2 a	92.6±0.3 a	94.4±0.9 a	94.7±1.1 a
Air volume (% *v*/*v*)	13.9±1.2 b	10.5±2.3 c	21.4±1.5 a	14.4±1.2 b
Water volume (% *v*/*v*)	79.8±1.1 a	82.1±2.1 a	73.0±1.5 b	80.5±1.1 a
Organic matter (% m/m)	95.9±0.2 a	95.8±0.0 a	96.8±0.4 a	94.4±0.2 b
pH (before liming)	4.8±0.05 a	4.3±0.01 a	4.5±0.01 a	4.6±0.01 a
pH (after liming)	6.2±0.12 a	6.5±0.04 a	6.1±0.13 a	5.9±0.24 a
EC (μS cm^{-1})[e]	41.1±0.2 b	88.9±1.6 a	89.2±0.4 a	40.4±2.0 b
Total carbon (%)	48.9±0.1 a	48.8±0.2 a	50.5±0.0 a	46.9±0.1 b
Total nitrogen (%)	1.04±0.1 a	1.02±0.1 a	0.90±0.0 b	0.87±0.1 b
Total C/total N	46.9±0.2 b	47.7±0.2 b	56.1±0.0 a	53.7±0.3 a
NH_4^+–N (mg L^{-1})	10.6±0.3 c	106.1±1.2 a	26.9±0.4 b	3.9±0.6 c
NO_3^-–N (mg L^{-1})	4.2±0.0 b	1.0±0.3 c	0.6±0.1 c	34.7±0.4 a

Values in a row followed by different letters are significant at $p<0.05$ (Tukey's test)

[a] According to Stewart and Kantrud (1971)

[b] Secondary particles from the humification process

[c] The mixtures of *Eriophorum*, small shrubs, soft and hard wood remains

[d] According to von Post (1924), where H1 stands for a completely undecomposed peat and H10 for a completely decomposed peat

[e] Electrical conductivity

and incubation temperature were similar to those used in the nitrate experiment.

Moisture experiment

To study the effect of WFPS on (N_2O+N_2)–N emission, triplicate subsamples (150 mL each) were amended with both KNO_3 and glucose at rates of 0.4 g N and 0.4 g C L^{-1} dry peat, respectively. Distilled and deionised water was added to achieve a moisture content of 40%, 50%, 60%, 70%, 80%, 90% or 100% WFPS. Acetylene was injected into the headspace of each jar (10% *v*/*v*) and incubated at 25°C. The headspace gas (10 mL each) was analyzed for (N_2O+N_2)–N at initial and after 1, 2, 3, 4, and 5 days.

Calculations and statistical analyses

In the glucose, nitrate and moisture experiments, the mean daily emission from a given treatment (μg L^{-1} dry peat day^{-1}) was estimated from the linear equation of measured (N_2O+N_2)–N vs incubation time. At each sampling time, there were three repeated measurements per treatment and peat type. The total emission over a given incubation period can, thus, be computed as the product of the mean daily emission and number of incubation days. Any increase in N_2O–N would represent (N_2O+N_2)–N from denitrification (Davidson et al. 1993) as the ammonium oxidation step of nitrification is effectively inhibited by acetylene even at low concentration (0.01–0.1% *v*/*v*). The daily emission (μg L^{-1} dry peat day^{-1}) in each treatment divided by the respective dry bulk density gives emission g^{-1} basis. The equation that best explains the relationship between (N_2O+N_2)–N emissions and glucose-C/NO_3^-–N ratio was chosen by considering the computed r^2 values and the standard errors of estimates. ANOVA was also performed and means were separated by the Tukey's comparison test ($p\leq0.05$) using the SAS software (SAS Institute, Inc. 9.1 for Windows, Cary, NC).

Results

Initial characteristics

The physicochemical properties and the botanical composition of peats are summarized in Table 1. All peats were composed of two or more plant genus (*Sphagnum*, *Carex*, *Bryales*, secondary particles, shrub, and woody plants) although the proportions were different from one peat to another. The degree of decomposition in these peats was H5 with the exception of the transitional peat that humifed less (H4). However, since the determination of humosity grade

is a subjective evaluation, these data may have a moderate reliability. The mesotrophic peat had the highest D_{BD} (109 g L^{-1}) and the eutrophic peat had the lowest (76 g L^{-1}) as the former peat contained the highest proportion of secondary particles (30% vs 10%). We assumed that secondary particles belong to the aforementioned plant groups although their determination was not possible due to their state of decomposition. Peats also differed significantly ($p<0.05$) in their air volumes (10.5–21.4% *v*/*v*), water volumes (73.0–82.1% *v*/*v*), and W_{om} (94.4–96.8% m/m) but were statistically similar ($p>0.05$) for P_S (92.6–94.7% *v*/*v*).

All peats had low initial pH (4.3–4.8 units; Table 1), and they were limed with $CaCO_3$ at a rate of 1.25 g L^{-1} dry peat. After a week of equilibration period, the final pH (in water) ranged from 5.9 to 6.5 units. The increase of pH in the mesotrophic peat was higher than the other peat types. Electrical conductivity in the mesotrophic and transitional peats was the same but statistically ($p<0.05$) higher than values of the other two peat types. Total C and total N ranged from 46.9% to 50.5% and from 0.87% to 1.04%, respectively, and the lowest amounts were measured from the eutrophic peat. The ratio of total C to total N in the transitional and eutrophic peats were significantly ($p<0.05$) higher than the corresponding ratio in the oligotrophic and mesotrophic peats. Ammonium-N in the mesotrophic peat (106.1 mg L^{-1} dry peat) was significantly higher than the rest of the peats (only 3.9–26.9 mg L^{-1} dry peat; Table 1). In contrast, the contents of NO_3^-–N in all but in the eutrophic peat were quite small.

Basal and potential denitrification rates

Basal denitrification rates were measured from both unlimed and limed subsamples without C and N inputs (Table 2). Emissions of (N_2O+N_2)–N from unlimed oligotrophic and transitional peats were statistically ($p<0.05$) lower than the corresponding values in the mesotrophic and eutrophic peats. These results revealed that the origin of the peats could greatly influence the basal denitrification rates despite the fact that all peats had similar initial pH (Table 1). After treating the bulk samples with $CaCO_3$, emissions of (N_2O+N_2)–N increased by 3.6- to 14-fold suggesting that the activity of denitrifying microorganisms in all peats was affected by the initial acidic pH.

Potential denitrification rates were measured from anaerobically incubated limed and unlimed subsamples after the additions of glucose-C and NO_3^-–N (Table 2). The lowest rate of (N_2O+N_2)–N emission was measured from unlimed transitional peat (119 μg L^{-1} dry peat h^{-1}) followed by oligotrophic (156), mesotrophic (189) and eutrophic peats (307). There appeared that the effect of liming on measured (N_2O+N_2)–N was positive and emissions increased by 1.4- to 2.3-fold compared with the corresponding values from the unlimed samples.

Nitrate experiment

Denitrification was measured from four contrasting peat samples amended with different levels of KNO_3 fertilizer with a constant addition of 0.4 g glucose-C L^{-1} dry peat (Fig. 1a). The mean daily emission of (N_2O+N_2)–N from the control (0 NO_3^-–N) treatment of the eutrophic peat was about 20 times higher than the other peat types. Emissions of (N_2O+N_2)–N from the oligotrophic, mesotrophic, and transitional peats were increased after the addition of 0.15 g NO_3^-–N L^{-1} dry peat although there were no appreciable increases for further NO_3^-–N inputs. However, the production of (N_2O+N_2)–N from the eutrophic peat was suppressed by the addition of NO_3^-–N. Overall, peats were ranked in the following descending order for the amounts of denitrified-N at all rates of NO_3^-–N inputs: eutrophic>mesotrophic>oligotrophic>transitional peat. Excluding the control samples from the computation, (N_2O+N_2)–N production in the eutrophic peat linearly increased by

Table 2 Emissions of (N_2O+N_2)–N (μg L^{-1} dry peat h^{-1}) from peat samples incubated under anaerobic condition. Basal denitrification rates were computed from glucose and NO_3^-–N unamended peat samples whereas the potential denitrification rates from amended ones

Peat types	Basal denitrification		Potential denitrification	
	Unlimed[a]	Limed[a]	Unlimed	Limed
Oligotrophic	2.0±0.3 c[b]	27.9±1.5 c	155.8±3.0 c	217.1±4.3 c
Mesotrophic	7.1±1.0 b	35.3±2.6 b	188.9±10.3 b	300.3±17.1 b
Transitional	2.0±0.6 c	17.6±3.4 d	118.8±4.2 d	272.4±25.8 b
Eutrophic	21.8±0.1 a	78.2±0.6 a	306.6±29.2 a	477.6±50.3 a

Values in a column followed by different letters are significant at $p<0.05$ (Tukey's test)

[a] See Table 1 for the pH value

[b] Mean ± standard deviation ($n=3$)

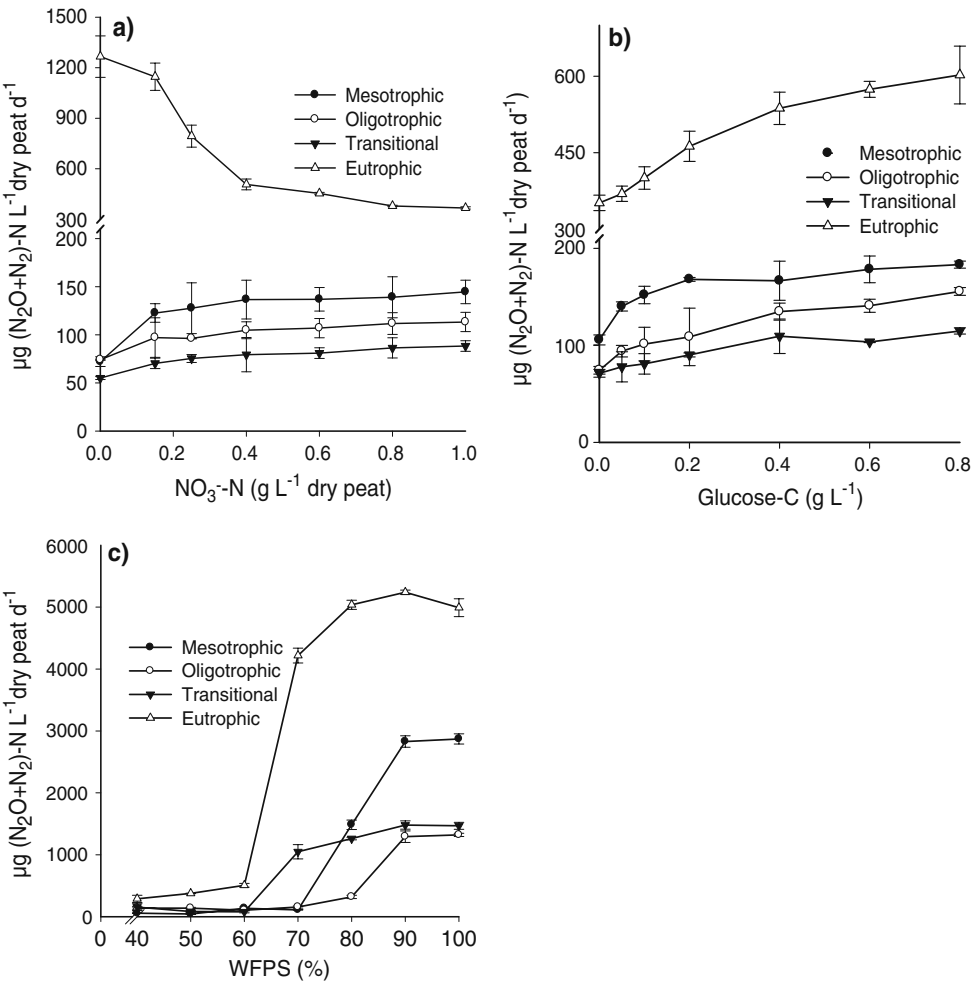

Fig. 1 Emissions of (N_2O+N_2)–N from four contrasting horticultural peats incubated at 25°C. **a** Samples treated with different concentration of NO_3^-–N with a blank addition of 0.4 g glucose-C L^{-1} dry peat and moisture content of 60% water-filled pore space (*WFPS*), **b** samples treated with varying concentration of glucose with a blank addition of 0.4 g NO_3^-–N and moisture content of 60% WFPS, and **c** samples treated with 0.4 g glucose-C and 0.4 g NO_3^-–N L^{-1} dry peat but differing in incubation moisture contents. Total emission from a given treatment can be computed from mean daily emission multiplied by incubation period (5 days). Each point was the mean of several measurements, and the *vertical bars* indicate standard errors

increasing the glucose-C/NO_3^-–N ratio (Fig. 2a) suggesting that the activity of denitrifiers was reduced by the amount of NO_3^-–N in the samples solution. In contrast, the relationships between denitrified-N and glucose-C/NO_3^-–N ratio in the oligotrophic, mesotrophic, and transitional peats were well explained by logarithmic relationships with negative slopes.

Glucose experiment

The daily (N_2O+N_2)–N concentration in the control (0 g glucose-C and 0.4 g NO_3^-–N) eutrophic peat was three to six times higher than the respective concentrations in the remaining three peat types (Fig. 1b). The rates of (N_2O+N_2)–N emission in the oligotrophic and transitional peats showed steep increases by adding glucose up to 0.4 g C L^{-1} dry peat although it happened only up to 0.2 g C L^{-1} in the mesotrophic peat. On average, (N_2O+N_2)–N emissions from the highest glucose amended samples (0.8 g C and 0.4 g NO_3^-–N) were 2.9, 2.0, 2.1, and 1.8 times higher than the respective emissions from the control treatments of mesotrophic, oligotrophic, transitional, and eutrophic peats. Denitrified-N in all peat types was positively and linearly correlated with the respective glucose-C/NO_3^-–N ratio (r^2> 0.86; Fig. 2b) although the relations seem an asymptotic curve.

Moisture experiment

Figure 1c shows the mean denitrified-N plotted against the sample moisture. Peats incubated at 40% or 50% WFPS were not statistically (p>0.05) different from each other but they differed (p<0.05) significantly for the higher moisture (60% to 100% WFPS). Both eutrophic and transitional peats showed sharp increases in (N_2O+N_2)–N when their respective moisture increased from 60% (507 and 79 μg L^{-1} dry peat day^{-1}) to 70% WFPS (4,217 and 1,049 μg L^{-1} dry peat day^{-1}). However, the oligotrophic peat required a moisture content of >80% WFPS to reach the highest rate of (N_2O+N_2)–N production. This peat had produced only 319 μg L^{-1} dry peat day^{-1} at 80% WFPS but the amount dramatically increased to 1,292 μg L^{-1} dry peat day^{-1} at 90% WFPS. The rate of (N_2O+N_2)–N emission in the mesotrophic peat presents a similar increase from 70%

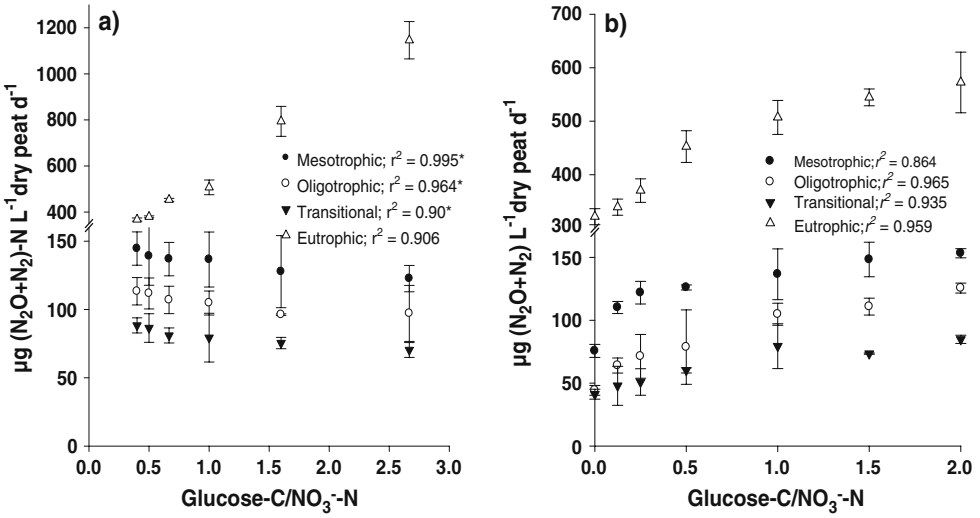

Fig. 2 Relationships between evolved (N_2O+N_2)–N and the ratio of glucose-C/NO_3^-–N in the samples at the time of incubation. **a** Samples treated with different concentration of NO_3^-–N for a fixed addition of 0.4 g glucose-C and **b** samples amended with different concentration of glucose-C and a blank addition of 0.4 g NO_3^-–N. All relationships are linear except for those marked with *asterisk* (logarithmic relationship). Total emission from a given treatment can be computed from mean daily emission multiplied by incubation period (5 days). Each point was the mean of several measurements, and the *vertical bars* indicate standard errors

(110 μg L^{-1} dry peat day^{-1}) to 80% WFPS (1,484 μg L^{-1} dry peat day^{-1}) or from 80% to 90% WFPS (2,829 μg L^{-1} dry peat day^{-1}). All peat samples at 90% WFPS reached roughly the same anaerobic condition as at 100% WFPS, since denitrification reached similar rates at the two WFPS (Fig. 1c). It was also observed that all peat types were visibly water logged at these WFPS.

Discussion

A basal denitrification rate measures the rate of denitrification based on the inherent soil properties (Drury et al. 1991). The highest basal denitrification rate was measured from the unlimed eutrophic peat and the lowest from the oligotrophic and transitional ones (Table 2). The observed variations in the basal denitrification rates were mostly explained by the peats water volume but not by air volume and total pore space. The highest emission in the eutrophic peat could, however, be explained by the presence of sufficient amount of NO_3^-–N (Table 1) to induce a higher denitrification activity compared with the other peat types. The eutrophic peat might also contain substantial amount of easily decomposable organic compounds that can be used as sources of electron donor by the heterotrophic denitrifiers. The eutrophic peat, as summarized by Shotyk (1988) and Steinmann and Shotyk (1997), is generally rich in nutrient and the aboveground biomass in this peat-forming environment decomposed regularly and allows the accumulation of easily decomposable plant materials in the peat. Results from the potential denitrification test (Table 2) seem to support the above two reasons where additions of glucose and NO_3^-–N increase emission in the eutrophic peat by only 14-fold as compared with the oligotrophic and transitional peats (78- and 60-fold, respectively).

Many of the physical, chemical, and biological properties and processes in soils are profoundly influenced by pH (Brady and Weil 1999). The initial pH in Table 1 was acidic and falls within the reported range for *Sphagnum*-dominated peats (Bohlin et al. 1989). The lower pH value in *Sphagnum*-originated peat is partly attributed to the presence of a relatively higher concentration of dissolved organic acids (Steinmann and Shotyk 1997). The production of gaseous N from such peats/acidic soils is generally lower than neutral or alkaline soils (Brady and Weil 1999). In this study, increasing the initial pH of the bulk samples from 4.3–4.8 to 5.9–6.5 increased (N_2O+N_2)–N emissions by 3.6–14-fold (in C and N unamended samples) or by 1.4–2.3-fold (in C- and N-treated peat samples) to confirm that the activity of denitrifying microorganisms was limited by low pH (Table 2). Similar results were reported by Rangeley and Knowles (1988), where denitrification rate was considerably increased by liming and glucose addition (pH=6.7 unit; 50 μg N_2O–N mL^{-1}) as compared with the unlimed but glucose received one (pH=3.5 unit; 2 μg N_2O–N mL^{-1}). Total (N_2O+N_2)–N emissions from the limed and C- and N-treated peat samples were about 0.70–2.15% of the added NO_3^-–N. Although we did not measure the relative contribution of denitrification to N_2O–N emission, this ratio is known to be affected by pH (Baggs et al. 2010; Brady and Weil 1999). Baggs et al. (2010), for instance,

identified denitrification as a predominant N_2O-producing process at pH 4.5 (in a fertilized loamy sand soil) and accounted for 80% of the ^{15}N–N_2O emitted over 41 days.

Nitrate fertilization does provide N substrates for denitrifiers (Bowman and Focht 1974; de Klein and van Logtestijn 1994; Myrold and Tiedje 1985) and influences (N_2O+N_2)–N emission to the atmosphere. Results from their studies indicated that the critical importance of NO_3^-–N concentration in the regulation of denitrification rate is approximated by a Michaelis–Menten mathematical function. According to Myrold and Tiedje (1985), denitrification rates would rarely be limited by the NO_3^-–N concentration greater than 20 mg kg^{-1} soil (i.e., >20 mg L^{-1} for soil with a mean dry bulk density of 1.0 kg L^{-1}). In this study, addition of 0.15 g NO_3^-–N L^{-1} dry peat sharply increases (N_2O+N_2)–N emissions from the oligotrophic, mesotrophic and transitional peats although no appreciable increases observed for further NO_3-N additions (Fig. 1a). However, the mean daily emissions of (N_2O+N_2)–N from the eutrophic peat decreased markedly with increasing NO_3^-–N concentrations. Although we did not expect a decrease in (N_2O+N_2)–N with increasing NO_3^-–N, high NO_3^- concentration in soil/pure culture reported to have an inhibitory effect on denitrification by inhibiting the activity of N_2O reductase (Gaskell et al. 1981) and NO_2^- and NO reductase (Payne and Riley 1969). We, therefore, assumed that the initial NO_3^-–N in eutrophic peat (34 mg L^{-1}; Table 1) might be sufficient for denitrifiers and for the induction of denitrifying enzymes.

The addition of easily decomposable C source could markedly increase the active denitrifiers biomass and thereby increases (N_2O+N_2)–N emissions (Myrold and Tiedje 1985). The apparent growth-related response after glucose addition is due to the fact that most denitrifiers in soils are chemoheterotrophs. Addition of glucose-C increased CO_2 evolution (data not shown) by decreasing O_2 availability, which may in turn increase denitrification rate. In this study, increased (N_2O+N_2)–N emissions with increasing glucose concentrations (Fig. 1b) generally indicating that the larger organic C pool in peats (>46%; Table 1) did not satisfy the immediate energy demand of heterotrophic denitrifying microorganisms. Although the glucose-C/NO_3^-–N ratio of 1 seems to be optimal for (N_2O+N_2)–N emissions, denitrification rates in all peat types showed positive linear relationships with glucose-C/NO_3^-–N ratio (Fig. 2b). The relationship between (N_2O+N_2)–N emission and the C/N ratio of inputs is, however, dependent on the type of materials used as C and N inputs. Additions of plant residues with a wide range of C/N ratio (8–118) into soil, for instance, resulted in a negative relationship with N_2O emission (Huang et al. 2004). N_2O–N emissions from black and grey Vertisol were decreased with increasing the amount of green waste compost (C/N=38.3) but increased with increasing feedlot manure (C/N=12) (Dalal et al. 2009). Earlier work by Bowman and Focht (1974) indicated that the C/N ratio necessary to effect maximal N_2O emission (via denitrification) could vary depending on the electron donors per mole of C substrate.

Since the moisture contents influence the availability and diffusion rate of oxygen in peat substrates (Agner and Schenk 2005), the effect of moisture (40–100% WFPS) on the production of (N_2O+N_2)–N was studied under laboratory conditions (Fig. 1c). The rates of denitrified-N at 40% or 50% WFPS were statistically similar between peat types. However, emissions at these relatively drier WFPS confirmed the existence of anaerobic microsites in the samples to favor denitrification activity. Denitrification rates are reported to increase with increasing water contents, especially when WFPS exceeds 60% (Groffman and Tiedje 1991). In this study, the WFPS of 60% or the corresponding water volumes of 56.2%, 55.6%, 56.6%, and 56.8% *v*/*v* were considered to be critical threshold limits in the oligotrophic, mesotrophic, transitional, and eutrophic peats, respectively, above which denitrification increased (but in different rates) with increasing water contents. Our results are comparable to Agner and Schenk (2005) and De Klein and van Logtestijn (1994) who found the critical threshold limits of 53% and 55% *v*/*v* for peat-based substrate and peat soil, respectively. However, the contributions of denitrification to the total N_2O emissions reported to be low at 60% WFPS (Agner and Schenk 2005; De Klein and van Logtestijn 1994; Pihlatie et al. 2004). For instance, Pihlatie et al. (2004) found that denitrification accounted for only 24%, 22%, and 33% in the peat, loamy sand, and clay soils, respectively, to suggest that gaseous N-loss from the tested peat samples would be high if N_2O emission from the nitrification process is considered. Emissions of (N_2O+N_2)–N from the eutrophic and transitional peats were started at lower WFPS compared with the oligotrophic peat to suggest that the tested peats should be irrigated differently so as to minimize gaseous N losses during crop cultivation.

Since we used unplanted peat materials, a direct comparison of our measured values to agricultural soils is somehow limited. However, Agner (2003) found that (N_2+N_2O)–N losses from horticultural pot plant production (using peat-based growing medium) were rather low and amounted to≈30% of emissions from agricultural system. This comparison was made on a hectare basis for a depth of 30 cm by considering 24 pots m^{-2}, three flooding events per week and a year-round production. However, both systems produced comparable N_2O–N losses to suggest that emission of environmentally harmful gas in horticultural pot plant production is considerably high. This might be due to high porosity of peat-based growing medium that allows a quick escape of gases from denitrifying sites.

In general, the acetylene inhibition technique (Yoshinari et al. 1977), which is adopted in this study and by Agner (2003), is known to underestimate the total loss of nitrate from the system (Bollmann and Conrad 1997) due to the formation of NO_2 from NO and acetylene in the presence of oxygen. In contrast to this, heterotrophic denitrifiers might use acetylene as a source of C. Despite its flaws, however, the acetylene reduction technique is one of the most widely used methods to measure denitrification from organic and inorganic soils because of its simplicity, acceptable sensitivity and low costs.

Conclusions

Basal and potential denitrification rates were greatly affected by the types of peat and the pH conditions. Addition of KNO_3 fertilizer at rate of 0.15 g N L^{-1} dry peat also markedly increased (N_2O+N_2)–N emissions from limed oligotrophic, mesotrophic, and transitional peats but further additions had no effect. The eutrophic peat, however, responded negatively to the added KNO_3 indicating that NO_3^-–N content of≈34 mg L^{-1} might be sufficient for denitrifiers and for the induction of denitrifying enzymes. An increase in (N_2O+N_2)–N emission from the glucose treated peat samples signifying the role of easily decomposable C sources for NO_3^-–N loss (via denitrification) during crop cultivation. The mean daily denitrification rates were also increased with increasing moisture contents to suggest that denitrification from fertilized potting media is favored by wetter conditions especially during and few hours after irrigation or heavy rainfall. A moisture content of 60% WFPS (≈56% *v*/*v*) was found to be a critical threshold limit in all peat types above which the emission of (N_2O+N_2)–N increased considerably. It can be concluded that conditions during plant production (liming, N-fertilization, availability of easily decomposable C and moist condition above 60% WFPS) could encourage the loss of NO_3^-–N through denitrification although the magnitudes greatly vary between peat types (eutrophic>mesotrophic>oligotrophic>transitional peat). We should also realize that the initial water–air dynamics in these peats might be changed during crop cultivation (due to settling and organic matter decomposition), which decrease the availability of oxygen and thereby increase N_2O emission.

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