Denitrification in cultures of potted ornamental plants

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Abstract

In horticultural production of potted ornamental plants N fertilizer is applied at high intensity. It was the aim of this research to investigate mechanisms and quantities of denitrification N loss from cultivation of potted ornamental plants.

Measurements were conducted with planted substrate in a dynamic system (flow-through chambers) and with unplanted substrate in a closed system (jars). N loss was determined as (N_2+N_2O)-N and as N_2O-N by use of the acetylene inhibition method. Substrate was planted with *Pelargonium zonale* 'Grand Prix' or *Euphorbia pulcherrima* 'Sonora Red' .

Denitrification in horticultural peat substrate proved to be mainly controlled by oxygen availability which decreased with increasing substrate water content. After flood irrigation, substrate water content was highest close to the pot bottom. Measurement of redox potential showed that N emissions originated from this substrate layer (up to 2.5 cm from the pot bottom). N emissions only evolved after irrigation events and ceased when mean substrate water content dropped below a threshold value. The decrease of water content was driven by evapotranspiration which increased with rising vapour pressure deficit (vpd) and plant size (transpiring leaf area). Thus, high substrate water content as well as denitrification N loss were favored by low vpd. Also, compaction or sieving of substrate, and use of bigger pots increased N loss per irrigation event. In contrast, N emissions and substrate water content decreased when flood irrigation was shortened.

Denitrification in planted and unplanted substrate was generally limited by carbon availability and increased after glucose-C amendment.

Rising nitrate supply consistently increased the share of N_2O emissions. (N_2+N_2O)-N loss, in contrast, was increased only relative to the unfertilized control treatment.

Further, sources of variability of N loss, the effects of plant age, substrate sieving, daytime of irrigation, pot design, and substrate composition on denitrification as well as the contribution of production surfaces to N emissions were discussed.

Summed up N loss in form of (N_2+N_2O)-N and N_2O-N from cultivation of potted plants amounted to 6.9 kg ha^{-1} $year^{-1}$ and 2.4 kg ha^{-1} $year^{-1}$, respectively. The economical and ecological importance of N emissions were evaluated and possibilities for restriction of denitrification in horticultural production were summarized.

Key words: denitrification, N loss, horticulture

Kurzfassung

In der gartenbaulichen Topfpflanzenproduktion erfolgt eine intensive N-Düngung. Es war das Ziel dieser Arbeit, Mechanismen und Mengen der N-Verluste durch Denitrifikation in getopften Zierpflanzenkulturen zu ermitteln. Messungen wurden mit bepflanztem Substrat in dynamischen Versuchssystemen (Durchflußkammern) und mit unbepflanztem Substrat in geschlossenen Gefäßen durchgeführt. N-Verluste wurden mithilfe der Acetylen-Inhibierungsmethode als (N_2+N_2O)-N und als N_2O-N bestimmt. Substrate wurden mit *Pelargonium zonale* 'Grand Prix' oder *Euphorbia pulcherrima* 'Sonora Red' bepflanzt.

In gärtnerischem Torfsubstrat wurde die Denitrifikation überwiegend durch die O_2 – Verfügbarkeit kontrolliert, die mit steigendem Substratwassergehalt sank. Nach Anstaubewässerung war der Substratwassergehalt am Topfboden am höchsten. Messungen des Redoxpotentials zeigten, daß N-Emissionen aus der untersten Substratschicht bis 2,5 cm über Topfboden stammten. N-Emissionen entstanden nur nach Bewässerungsereignissen und endeten, wenn der Substratwassergehalt einen Schwellenwert unterschritt. Das Absinken des Wassergehalts wurde durch die Evapotranspiration angetrieben, die mit Anstieg von Wasserdampfdruckdefizit (vpd) und Pflanzengröße (transpirierender Blattfläche) zunahm. Somit wurden ein hoher Substratwassergehalt sowie N-Verluste durch Denitrifikation durch ein geringes vpd gefördert. Ebenso erhöhten die Verdichtung oder Siebung von Substrat, sowie die Verwendung größerer Töpfe die N-Emissionen pro Bewässerung. Wassergehalt und N-Verluste verminderten sich dagegen mit abnehmender Anstaudauer.

In bepflanztem und unbepflanztem Substrat war die Denitrifikation im allgemeinen Kohlenstoff-limitiert und wurde durch Gabe von Glukose-C erhöht. Ein steigendes Nitratangebot erhöhte den N_2O-Anteil der N-Emissionen. Der (N_2+N_2O)-N-Verlust wurde dagegen nur im Vergleich zur ungedüngten Kontrolle gesteigert. Desweiteren wurden Ursachen der Variabilität von N-Emissionen, Wirkungen von Pflanzenalter, Tageszeit der Bewässerung, Topfart und Substratzusammensetzung auf die Denitrifikation, sowie der Beitrag von Produktionsflächen zu N-Verlusten diskutiert.

Der hochgerechnete N-Verlust als (N_2+N_2O)-N und N_2O-N aus Topfpflanzenkulturen betrug 6,9 kg ha^{-1} $Jahr^{-1}$ bzw. 2,4 kg ha^{-1} $Jahr^{-1}$. Die ökonomische and ökologische Bedeutung der N-Emissionen wurde diskutiert und Möglichkeiten für die Begrenzung der Denitrifikation in der gartenbaulichen Produktion wurden zusammengefaßt.

Stichwörter: Denitrifikation, N-Verlust, Gartenbau

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Abbreviations / Abkürzungen

C	carbon	Kohlenstoff
CH_4	methane	Methan
C_2H_2	acteylene	Acetylen
C_2H_4	ethylene	Ethylen
CO_2	carbon dioxide	Kohlendioxid
d.m.	dry matter	Trockenmasse
f.m.	fresh matter	Frischmasse
ha	hectare	Hektar
h	hour	Stunde
H_2O	dihydrogenoxide, water	Wasser
HCl	hydrochloric acid	Salzsäure
LSD	least significant difference	Grenzdifferenz
N	nitrogen	Stickstoff
N_2	molecular nitrogen	molekularer Stickstoff
NH_4	ammonium	Ammonium
NO	nitric oxide	Stickstoffmonoxid
N_2O	nitrous oxide	Distickstoffoxid, Lachgas
NO_3	nitrate	Nitrat
O	oxygen	Sauerstoff
O_2	molecular oxygen	molekularer Sauerstoff
vppm	volume parts per million	1:1 Million (Volumeneinheit)
vppb	volume parts per billion	1:1 Milliarde (Volumeneinheit)
PVC	polyvinyl chloride	Polyvinylchlorid, Kunststoff
rh	relative humidity	relative Luftfeuchte
T	temperature	Temperatur
vol.%	volume percent	Volumenprozent
vpd	vapor pressure deficit	Wasserdampfdruckdefizit
vppm	volume parts per million	1:1 Million (Volumeneinheit)

1. Introduction

Ornamental pot plants are generally produced in most intensive production systems characterized by high fertilizer input and frequent irrigations. Yet, only little information exists on denitrification N loss from horticultural growing systems based on peat substrate.

In greenhouse production of cucumber plants in a soilless cultivation system, mean N losses due to denitrification amounted to about 180 kg N ha^{-1} $year^{-1}$ (Daum and Schenk 1996). In intensive field production of vegetables high denitrification N loss of up to 5 kg ha^{-1} day^{-1} was observed after incorporation of crop residues (Schloemer 1991), and N loss up to 2 and 3.6 kg ha^{-1} day^{-1} was reported from vegetable fields after irrigation or rainfall events, respectively (Ryden and Lund 1980). Also, highly fertilized grassland soils denitrified up to 3 kg N ha^{-1} day^{-1} under moist conditions following fertilizer application (De Klein and Van Logtestijn 1996).

In all of these cases, N emissions were especially high because the conditions for denitrification were extraordinarily favorable. Availability of oxygen was low due to irrigation or rainfall events or oxygen consumption by root and microbial respiration. Supply of nitrate was high due to intensive fertilization, and so was availability of easily decomposable carbon derived from roots or from incorporation of organic material.

In production of potted ornamental plants, conditions for denitrification were assumed to be comparably favorable. Frequent irrigation events could be expected to induce oxygen deficiency in the substrate, high availability of nitrate was granted by fertilization or fertigation, and easily available carbon was likely to be supplied by plant roots or by the growing medium itself. Consequently, high denitrification N loss was expected from this intensive production system.

Oxygen deficiency, availability of nitrate and of carbon are the key factors of biological denitrification, which is the reduction of mineral nitrogen oxides (nitrate and nitrite) to N gases (NO, N_2O, N_2) by microbes. The main end products are molecular nitrogen (N_2) and nitrous oxide (N_2O), while the formation of nitric oxide (NO) during denitrification is considered to be comparatively low (Davidson 1993).

The reduction of N-oxides occurs mainly under conditions of oxygen deficiency when facultative anaerobic bacteria use nitrate instead of oxygen for electron transfer (Tiedje et al. 1989). Because of higher energy yield from oxygen relative to nitrate reduction nearly all respiratory denitrifiers prefer to use O_2 as electron acceptor and only reduce N-oxides when O_2 is not available (Tiedje et al. 1989). In soils oxygen deficiency was often reported to be induced by rainfall or irrigation events, which resulted in increased emission of N gases from soil. Emissions ceased when soil water content decreased and air and oxygen returned into soil pores. The degree of oxygen deficiency is affected by soil physical properties and higher N emissions were observed e.g. from compacted or fine textured soils (Torbert and Wood 1992, Bakken et al. 1987, Aulakh et al. 1991b, Sexstone et al. 1985a).

In addition to oxygen deficiency, denitrification requires availability of nitrate (electron acceptor), which is generally granted in cultivated soils. Only in natural ecosystems denitrification is considered to be possibly limited by low nitrate availability (Myrold and Tiedje 1985).

Readily available carbon is the source of energy for the reduction process (electron donator). It has shown high impact on the intensity of denitrification, e.g. after incorporation of crop residues (Schloemer 1991, Aulakh et al. 1991b) indicating that its availability is often limiting denitrification in cultivated soils. Easily decomposable carbon compounds may also be supplied by plant roots (Hütsch et al. 2002). Yet, denitrification was not always reported to be increased by the presence of plants (Haider et al. 1985, Haider et al. 1987, Qian et al. 1997).

Additionally, denitrification is stimulated by high temperature (Stanford et al. 1975, Dobbie and Smith 2001), which increases microbial activity and accelerates oxygen consumption.

Denitrification is considered to play a central role in the N cycle as it seems to balance nitrogen fixation by returning fixed nitrogen to the atmosphere (Tiedje et al. 1989, Aulakh et al. 1992). Yet, it is of concern to agronomists and environmentalists because it causes loss of N fertilizer and it is also one of the most important sources of environmentally harmful nitrous oxide (N_2O).

N_2O emissions are considered to effectively contribute to global warming and to the destruction of the ozone layer. In the troposphere, which reaches up to about 15 km from the Earth's surface, N_2O among other gases (e.g. CO_2, CH_4, H_2O) is

responsible for the absorption of infrared radiation emitted by the Earth. This absorbed thermal radiation is partly sent back to the Earth leading to the surface warming which is necessary for life. Yet, increasing concentrations of infrared absorbing gases in the troposphere may lead to increased surface heating resulting in changes of climate (Mackay and Khalil 2000, Linak and Kramlich 1997). Although concentrations of N_2O are very low (about 310 vppb), its global warming potential is estimated to be about 300 times that of CO_2 over a 100 year horizon, which is due to the long atmospheric lifetime of N_2O of more than 100 years (IPCC 2001, Gale et al. 2000). The contribution of N_2O to global warming is considered to be 5-6 % (IPCC 1996, Rodhe 1990). In the stratosphere, which extends from about 15 to 50 km above the Earth's surface, N_2O plays an important role in the destruction of the ozone layer which protects our biosphere from harmful ultraviolet radiation (UV-B) (Aulakh et al. 1992, Crutzen 1981). There, photolysis of N_2O leads to formation of excited oxygen (O) which reacts with nitrous oxide (N_2O) to form nitric oxide (NO). Presence of NO leads to destruction of ozone (O_3) as both react to form nitrite (NO_2) and oxygen (O_2). In contrast to ozone, NO is recycled by reaction of NO_2 with O and it then returns to destroying O_3. The net contribution of N_2O to ozone destruction is not clear, yet it presumably is much lower than that of industrial compounds, like chlorofluorocarbons, hydrochlorofluorocarbons or halons (Crutzen 1981, IPCC 2001).

The contribution of soils to emission of N_2O is considered to be high. According to Bouwman et al. (1995) two-thirds of global N_2O emissions originate from natural and cultivated soils, where N_2O is formed mainly during microbial nitrification and denitrification. The contribution of agriculture alone to global N_2O flux amounts to 40 % (Oenema et al. 2001) and its share among anthropogenic sources of N_2O was estimated to be about 80 % (Gale et al. 2000, Isermann 1994).
Denitrification has been thoroughly investigated in laboratory and field studies. But still, much uncertainty exists in the assessment of nutrient loss and N_2O production (Kroeze et al. 2003). This is mostly due to the many variable and interacting factors controlling not only total denitrification N loss but also the ratio N_2O:N_2 of N emissions (Firestone et al. 1979, Weier et al. 1993, Beauchamp 1997). Temporal variability of N emissions was attributed to temperature changes in soil during day (Blackmer et al. 1982), to differences in time required for induction of anaerobiosis within a soil volume (Christensen et al. 1990b), and to the development of soil moisture and plant

growth over the growing period (Rudaz et al. 1999). Spatial variability of emissions was traced back to locally limited sources of easily available carbon (Christensen et al. 1990a, Parkin 1987). Inhomogeneous distribution of carbon and nitrate, and only temporary occurrence of oxygen deficiency are common in soils as well as shifts in temperature over time. Consequently, variation of N emissions is usually high. Folorunso and Rolston (1984), for example, calculated variation coefficients from 161 % to 508 % for (N_2+N_2O)-N emissions from a 3 m x 36 m area of field soil.

Because of the many variable factors, accurate prediction of denitrification N loss and N_2O emissions by modeling has not yet been achieved (Kaiser et al. 1996, Frolking et al. 1998, Parton et al. 1996, Potter et al. 1997). Measurement still seems the most reliable way of quantifying denitrification N loss at the small scale.
Thus, it was the aim of this study to investigate denitrification by measurement of gaseous N loss from cultures of potted ornamental plants. Within this study it was intended

- to develop and adjust an assay system for measurement of N_2 and N_2O emissions from potted plants,
- to study factors controlling denitrification in unplanted horticultural substrate,
- to investigate dynamics of denitrification in planted substrate,
- to determine the effects of plant age and carbon supply on denitrification in planted substrate,
- to investigate the effects of substrate physical characteristics on denitrification,
- to localize denitrifying zones in potted and planted substrate,
- to determine the influence of horticultural management practices on denitrification, and finally
- to estimate the dimensions and to evaluate the economical and ecological importance of N_2 and N_2O emissions due to denitrification from horticultural pot plant production.

2. Assay system for measurement of denitrification

2.1 Introduction

Information on denitrification N loss from horticultural production systems is rare in literature, since research has focused on agricultural production. However, for vegetable production in the field and in soilless culture it was shown that gaseous N losses due to denitrification may reach from 0.024 to 5.2 kg N ha^{-1} day^{-1} (Ryden and Lund 1980, Schloemer 1991, Daum and Schenk 1996) compared to 0.002 to 0.42 kg N ha^{-1} day^{-1} in agricultural production (Ryden et al. 1979b, Kaiser et al. 1996). Reasons for the high denitrification potential in horticultural cropping systems are the more favorable conditions which are mainly due to high availability of carbon and nitrate, and frequently induced oxygen deficiency by irrigation. As fertilizer and irrigation are also intensively used in horticultural production of potted ornamental plants this production system was considered a potential high producer of denitrification N gases.

To investigate denitrification N loss from potted ornamental plants, it was intended to use the acetylene (C_2H_2) inhibition technique. This method has extensively been used in field and lab experiments and was often described as an inexpensive, comparably simple, and sensitive method for quantification of denitrification N loss (Gross and Bremner 1992, Duxbury 1986, Keeney 1986, Tiedje et al. 1989, Yoshinari et al. 1977). The underlying mechanism of this technique is the inhibition of N_2O reduction to N_2 in presence of C_2H_2 (Yoshinari et al. 1977). In consequence, the final product of nitrate reduction is N_2O which in contrast to N_2 can readily be measured by gas chromatography. Additionally, the production of N_2O in absence and presence of C_2H_2 allows calculation of the probable mole fraction of N_2O in denitrification products (Yoshinari et al. 1977).

Yet, application of C_2H_2 is not free of difficulties. An often described problem is the failure of C_2H_2 inhibition because of insufficient C_2H_2 distribution throughout the soil (Rolston 1986, Duxbury 1986, Tiedje 1988). Also, C_2H_2 application may lead to secondary effects through contaminants like acetone or ethylene (C_2H_4) (Hyman and

Arp 1987), which is a plant hormone and may change conditions for denitrification by affecting the plant.
To trap gases produced by denitrification in soil with or without application of C_2H_2 soil covers or chambers are installed in the field (Aulakh et al. 1991a, Hutchinson and Livingston 1993). Different chamber systems are discussed in literature, e.g. closed or open chambers with or without venting and with or without air exchange (Hutchinson and Mosier 1981, Jury et al. 1982, Ambus et al. 1993, Hutchinson and Livingston 1993). Furthermore, in chambers that do not include the plant shoot measurements may be biased by losses of N_2O to the atmosphere through N_2O transport and emission by the shoot (Ferch and Römheld 2001, Chang et al. 1998). While it is unanimously agreed upon the importance of the experimental setup for the quality of measurement, recommendations on methodology differ.

So, before denitrification N loss itself could be measured from potted ornamental plants, the experimental setup had to be developed to guarantee nonbiased experimental results. Mainly three issues are discussed in this paper: 1. the application of acetylene for inhibition of N_2O reduction, 2. side effects of C_2H_2 on plants, and 3. the choice of the experimental system, i.e. static (closed container) or dynamic (flow-through chamber).

2.2 Materials and methods

2.2.1 Experimental setup for denitrification measurement

2.2.1.1 Closed system

For denitrification measurement in a closed system 1.5 L glass jars (Weck) were used. The jars were closed by glass lids and rubber seals between rim and lid guaranteed air thightness. The lids were fixed to the jars by metal clips. To allow addition and withdrawal of gas from the jars septums were inserted into the lids.
When planted substrate was investigated the complete plant (including 340 mL-plastic pot, substrate and shoot) was closed into a jar like shown in Figure 1.

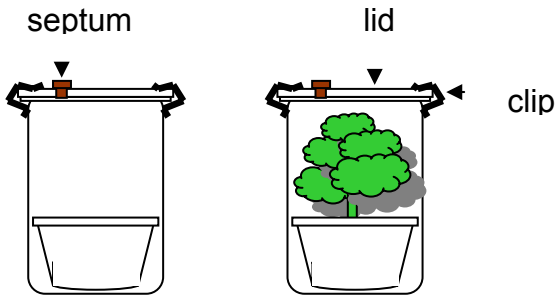
Unplanted substrate was evenly filled into 280 mL plastic pots according to its weight and pots were then put into jars.

Before the start of the experiment substrates were pretreated with acetylene (C_2H_2) within the jars as described in Chapter 2.2.3.

After pretreatment with C_2H_2 jars were opened and pots were flood irrigated within the jars with 100 mL or 150 mL of a solution containing 150 mg NO_3-N L^{-1}. The amount of solution depended on pot size and water content of the substrate. It was uniform among replications of each experiment.

After addition of nutrient solution the jars were closed, C_2H_2 was renewed and incubation at 26°C started (growth chamber). Air samples were taken with syringes from the jars after 24 h and 48 h of incubation. Only in the experiment comparing closed vs. dynamic system jars were incubated in a greenhouse and sampled at shorter time intervals (after 12, 24 and 30 hours of incubation).

Fig. 1 Experimental setup for denitrification measurement using glass jars



2.2.1.2 Flow-through chamber

Flow-through chambers adapted from Daum and Schenk (1997) were used for measurement of denitrification in a dynamic system. The PVC-chambers had a volume of 12 L and were provided with openings for air exchange like shown in Figure 2. One opening allowed the inflow of air or of air amended with C_2H_2. Another opening was connected to a pump which sucked air out of the chamber at a rate of 50 L h^{-1}, thus providing a fourfold air exchange per hour. The air flow was controlled by a flowmeter which was protected against air humidity by a molecular sieve.

The lid of each chamber had a hole in its center and was divided by half into one fixed and one replaceable part which allowed opening. Planted pots (volume 340 mL) were positioned so that the plant stem went through the hole in the lid and the shoot remained outside the chamber. Then the opening around the stem was sealed to avoid escape of N_2O and the removable lid was fixed with adhesive tape to the chamber.

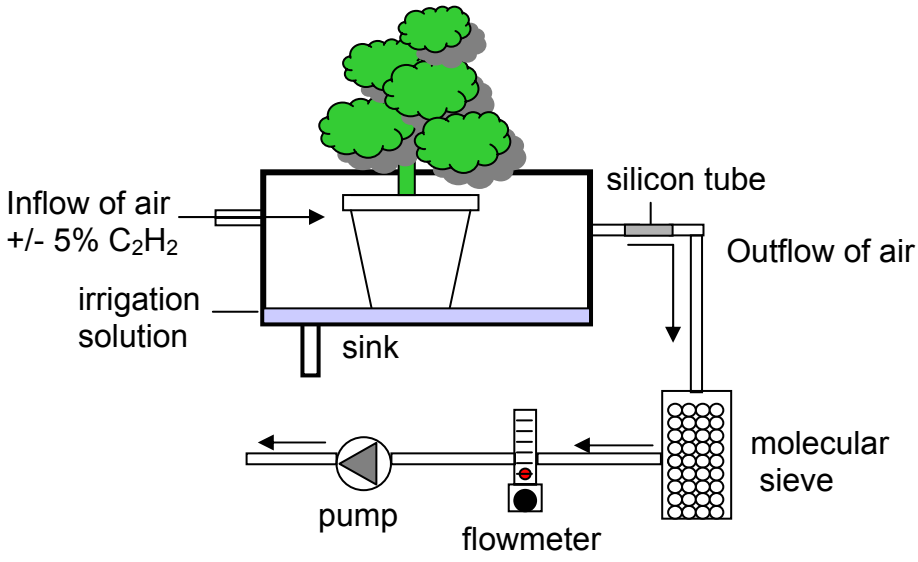
Unplanted substrate was evenly filled into 280 mL plastic pots and closed into the chambers. The hole in the lid was sealed with adhesive tape during measurement.

Before the start of the experiment substrates were pretreated with acetylene (C_2H_2) within the chambers as described in Chapter 2.2.3.

After pretreatment with C_2H_2 pots were flood irrigated within the chambers with 1 L of a solution containing 150 mg NO_3-N L^{-1}. Two hours later, the irrigation solution was released by an opening at the bottom of the chambers.

The beginning of the irrigation event was defined start of the experiment. Air samples were taken with syringes topped with gauge needles from silicon tubes at the air outlet of the chambers every one to two hours until N_2O emissions ceased.

Fig. 2 Experimental setup for denitrification measurement using flow-through chambers



2.2.2 Experimental setup for measurement of N_2O release by the plant shoot

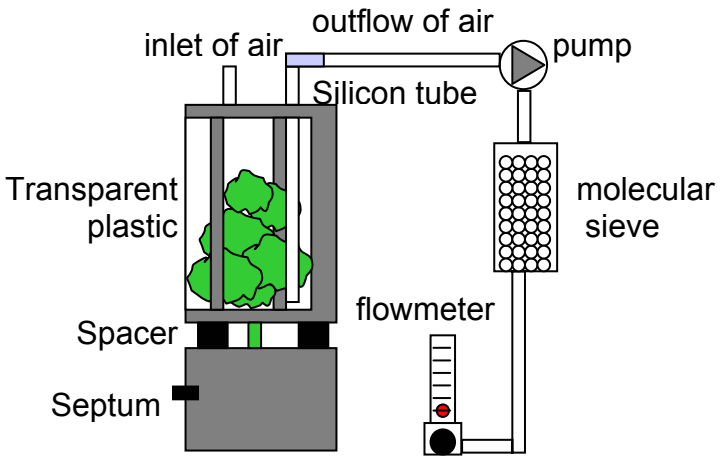
Plants of *P. zonale* and *E. pulcherrima* were used for measuring N_2O release by the shoot. The pots of the plants were watered and then locked in small air tight plastic chambers made of PVC tubes and tube end pieces. The shoots were closed into chambers made of long tubes and transparent film like shown in Figure 3.

While the lower compartment was meant to be closed to the atmosphere, the upper one was provided with an aeration system to control air humidity and thus allow transpiration and possibly N_2O transport. The air exchange rate was adjusted to 120 L h^{-1} by a flowmeter which was protected against air humidity by a molecular sieve.

At the beginning of the experiment, pure N_2O was added through a septum to the root chambers to reach concentrations of 0, 5, 50 and 500 vppm. Acetylene was added at 5 vol.% to avoid N_2O reduction. During the experiment N_2O concentrations in the root compartments were checked every 1.5 hours and corrected if necessary.

Air samples were taken with syringes from the outflow of the shoot chambers every 1.5 hours.

Fig. 3 Experimental setup for measurement of N_2O release by the plant shoot



2.2.3 Application of acetylene (C_2H_2)

C_2H_2 was applied for measurement of (N_2+N_2O)-N loss by denitrification. To guarantee immediate inhibition of N_2O reductase since the start of each experiment, substrates were pretreated with C_2H_2 before irrigiation. In general the pretreatment with C_2H_2 lastet two hours. It differed only in two experiments dedicated to the determination of the optimum pretreatment duration. In one jar experiment substrate was pretreated with 5 vol.% C_2H_2 for 0, 2, 12, and 24 hours, respectively. In a flow-through chamber experiment with planted substrate (*P. zonale*) pots were pretreated with 5 vol.% C_2H_2 for 12 h and 2 h, respectively.

For determination of the optimum C_2H_2 concentration a jar experiment with unplanted peat substrate was conducted. Increasing concentrations of C_2H_2 (0, 1, 3 or 10 vol.%) were added to the jar atmosphere at the beginning of the pretreatment and of the incubation which started after irrigation. In all other experiments 5 vol.% C_2H_2 were applied.

To reduce the acetone content of C_2H_2 gas emerging from a cylinder the gas was lead through two water traps (10 L and 2 L) in flow-through chamber experiments (Gross and Bremner 1992). The water of the traps was exchanged every six hours.

2.2.4 Substrates

Two kinds of unplanted substrate were used for experiments: a sieved peat substrate (5 mm) which had been used for cultivation of plants and contained decomposable organic matter (root residues), and a peat substrate amended with 1 mg L^{-1} dried and ground organic matter (*Lolium westerwoldicum*) to insure microbial activity. Both substrates were adjusted to pH6 and fertilized with a complete compound fertilizer. Only one of these substrates was used per experiment.

In denitrification measurement planted substrate consisted of sieved peat (5 mm) which was adjusted to pH6 and fertilized with a complete compound fertilizer prior to planting. During cultivation plant (and substrate) were fertigated according to horticultural practice.

Plants that were used for measurement of N_2O release by the shoot were grown in a commercial, fertilized, peat based substrate at pH6.

2.2.5 Plant material

Plants of *Pelargonium zonale* 'Grand Prix' and *Euphorbia pulcherrima* 'Sonora Red' were used for experiments. Plants were propagated by cuttings and rooted in small peat nuggets (Jiffy7) for two weeks. Then, plants were potted into 340 mL pots and cultivated for at least four weeks to guarantee rooting of the substrate.
For the pretreatment experiment and for comparison of dynamic vs. static incubation system pots of *P. zonale* were used while N_2O release from the shoot was measured from both species, *P. zonale* and *E. pulcherrima*.
For each experiment plants of the same set were used, i.e. plants were of the same age and of similar growth.

2.2.6 Analytical procedures

2.2.6.1 N_2O

The analysis of N_2O in all air samples was performed by a gas chromatograph (Chrompack 9001) with an electron capture detector (ECD) according to a method described by Mosier and Mack (1980).

2.2.6.2 C2H2

C_2H_2 was measured by means of a gas chromatograph (Carlo Elba) equipped with a flame ionization detector (FID).
Plant damage by C_2H_2 application was scored by counting chlorotic, necrotic, and healthy leaves per plant after 0, 24, 48, 72, and 96 hours of treatment.

2.2.6.3 Evapotranspiration and substrate water content

Evapotranspiration was determined by weighing of planted pots every 12 hours for two days starting from irrigation and calculating decrease in weight over time. The shoot fresh matter was determined by cutting off and weighing of the plant shoot. It allowed calculation of evapotranspiration per unit shoot fresh matter.

2.2.6.4 Carbon content of substrate

To investigate the effect of C_2H_2 application on carbon content of the substrate plants of *P. zonale* and *E. pulcherrima* were grown in fine-grained quartz sand for six weeks and treated for 24 hours with air and air + 5 vol.% C_2H_2, respectively. For extraction of carbon from the pots, 200 mL A. dest. were dripped onto the sand surface. The eluate was collected in polyethylene bottles and mixed with 2 mL HCl to avoid microbial digestion of C until analysis. The plants were harvested, root and shoot fresh matter were determined. The carbon content of the samples was analyzed by use of a total carbon analyzer (GO-TOC 100, Ultramat 5E - 2R, Fa. Gröger und Obst (Starnberger See)).

2.2.7 Statistics

Statistic calculations were performed by use of the SAS package. The number of replications for each treatment was usually five. Only the experiments comparing evapotranspiration +/- C_2H_2 treatment and closed vs. dynamic system had six replications in each treatment.

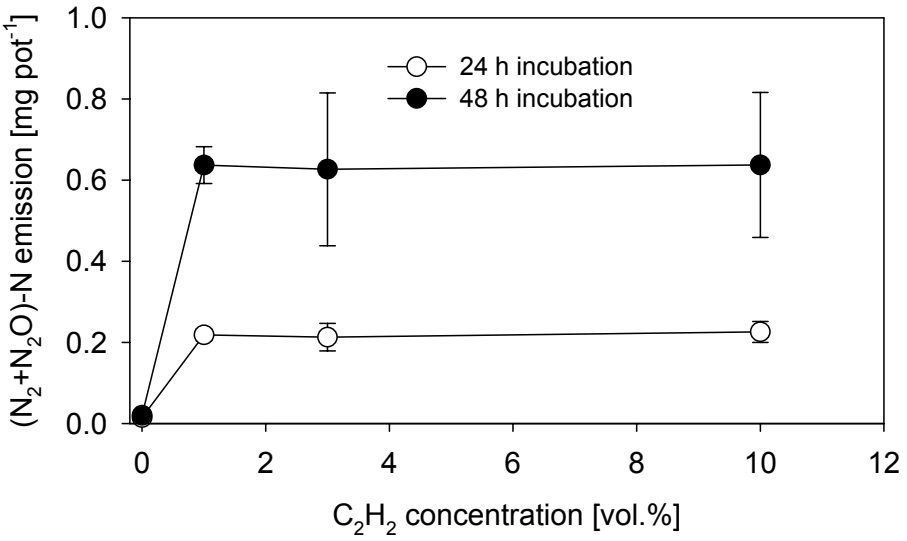
2.3 Results

2.3.1 Application of acetylene (C_2H_2)

2.3.1.1 Optimum concentration of C_2H_2

Figure 4 shows the amounts of N_2O-N emitted from unplanted peat substrate at different C_2H_2 concentrations. Without addition of C_2H_2 measurable N emissions were very low. Independent of the incubation period, adding only 1 vol.% acetylene to the jar atmosphere already resulted in a significantly higher N_2O accumulation and further increasing the C_2H_2 concentration up to 10 vol.% did not yield higher N_2O emissions. A concentration of 5 vol.% acetylene was chosen for further experiments.

Fig. 4 N_2O-N emissions from unplanted peat substrate as affected by C_2H_2 concentration after 24 h / 48 h of incubation following flood irrigation (closed system)



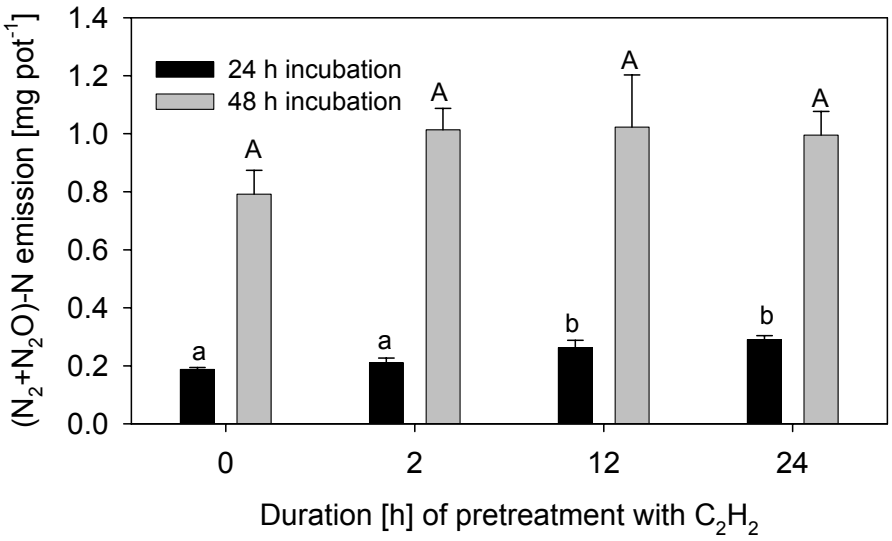
2.3.1.2 Optimum duration of C_2H_2 pretreatment

The effect of increasing durations of C_2H_2 pretreatment of unplanted substrate on the recovery of N_2O was investigated (Fig. 5). After 24 h of incubation in a closed system there was a significant difference between the treatments 0 h and 2 h on one hand, and treatments 12 h and 24 h on the other hand. However, after 48 h of incubation there was no significant influence of pretreatment duration on N emissions.

Additionally, the effect of C_2H_2 pretreatment on N emissions was examined with planted substrate (*P. zonale*) in flow-through chambers. In this system no significant difference in N emissions could be observed between the 2 h and 12 h treatment (Tab. 1), although the measurement lastet only 30 hours. This was contradictory to results obtained with the closed system after 24 hours of incubation (Fig. 5). The mean N losses per pot in flow-through chambers were comparable to the emissions found in the closed jar experiment after 24 h of incubation but the standard deviation in the flow-through chamber experiment was remarkably higher.

Although these results do not prove its necessity, a pretreatment of 2 hours was chosen for the following experiments in order to exclude failure of C_2H_2 inhibition.

Fig. 5 N_2O-N emissions from unplanted peat substrate as affected by duration of pretreatment with 5 vol.% acetylene C_2H_2 after 24 h and 48 h of incubation following irrigation (closed system)



*Differing letters indicate statistically significant effects between treatments (pairwise comparison; α=0.05)

Tab. 1 N_2O-N emissions from pots of *P. zonale* in flow-through chambers with 2 h or 12 h of pretreatment with 5 vol.% C_2H_2

C_2H_2 pretreatment	N_2O-N emission [mg pot^{-1}]
2 h	0.253 a*
12 h	0.214 a*

*Differing letters indicate statistically significant effects (t-Test, α=0.05)

2.3.2 Side effects of C_2H_2

2.3.2.1 Visible symptoms of C_2H_2 application on plants

The application of C_2H_2 lead to visible symptoms on the plants. *P. zonale* showed chlorosis on all leaves after 48 hours of C_2H_2 application (Fig. 6, 7b). At 72 hours leaves started to develop necrosis and at 96 hours 2/3 of the plants were severely damaged. *E. pulcherrima* plants showed no chlorosis at all and only little necrosis

during C_2H_2 exposition. Instead, the leaves slightly bended downwards which was reversible at first and disappeared after a few days in C_2H_2 free air. After five days of C_2H_2 treatment leaves of *E. pulcherrima* wilted and curled (Fig. 6, 7a).

Fig. 6 Leaf damage [%] on five plants of *P. zonale* and *E. pulcherrima* caused by treatment with 5 vol.% C_2H_2 as affected by duration of treatment (flow-through system)

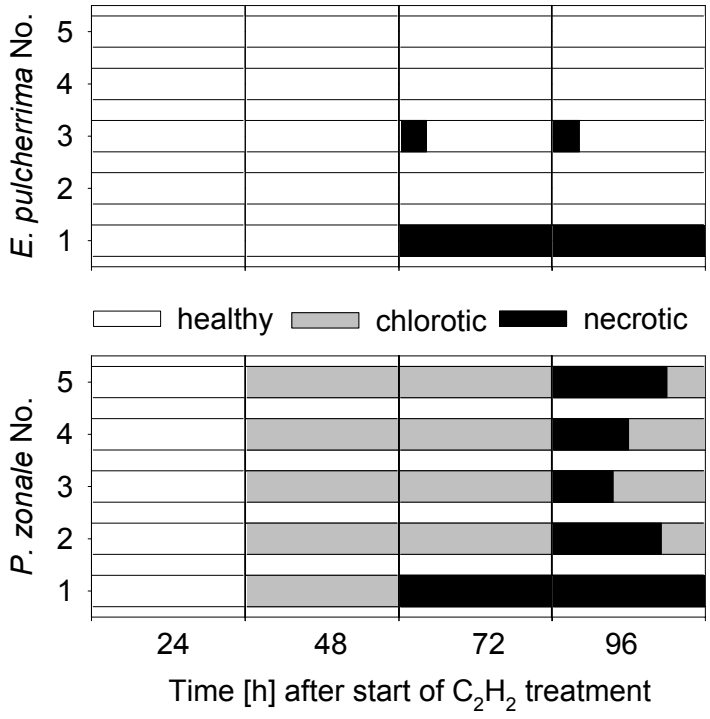
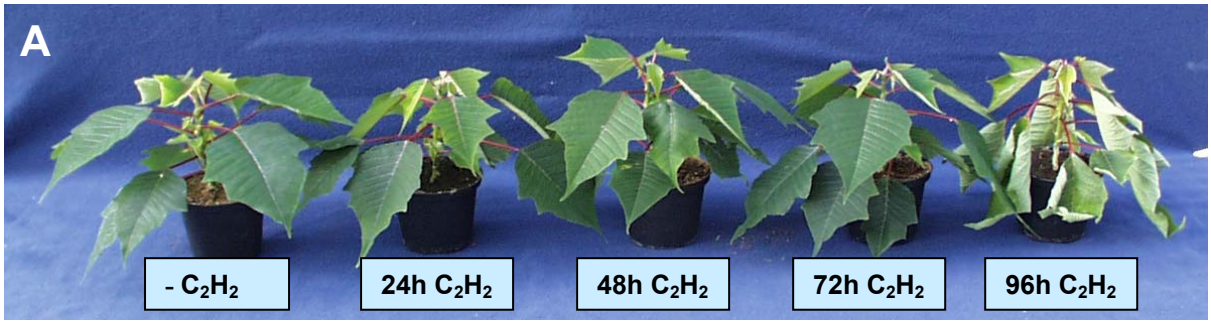
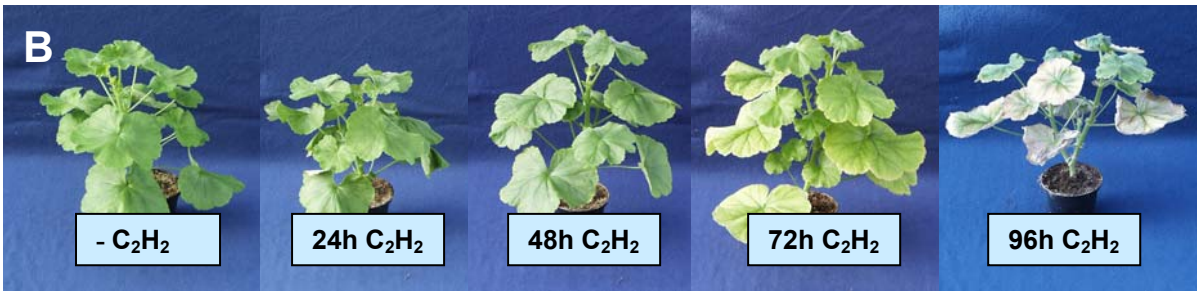


Fig. 7 Leaf damage of *E. pulcherrima* (A) and *P. zonale* (B) depending on duration of treatment with 5 vol.% C_2H_2

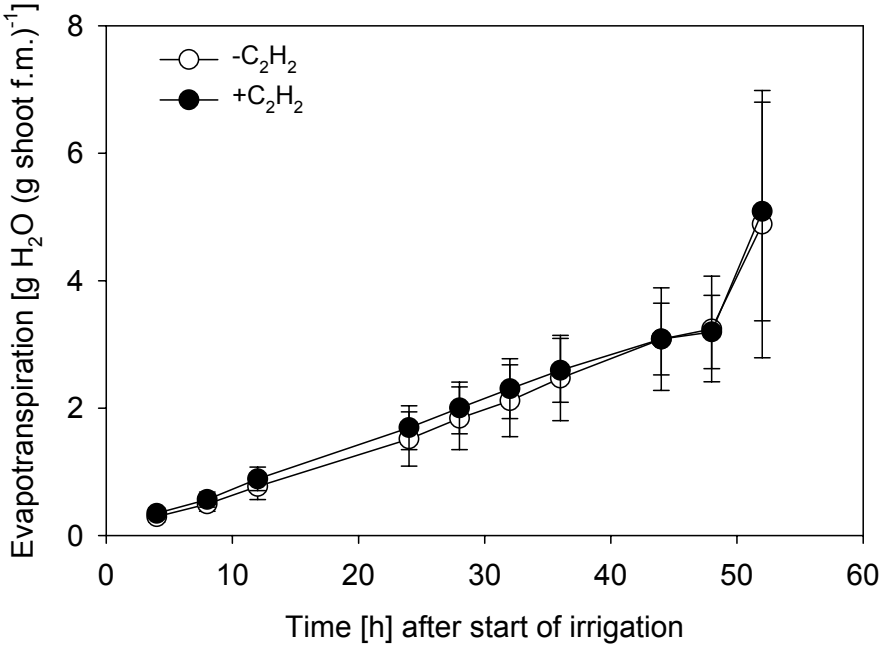




2.3.2.2 Water uptake and transpiration as indicator of root integrity

The amounts of water taken up from the substrate and transpired by the plants are presented in Figure 8. No difference could be demonstrated with regard to 52 hours of C_2H_2 treatment, although the plant shoots had already reacted by this time and showed chlorosis. Still, sometimes it was observed that after prolonged application of C_2H_2 substrate contained more water than untreated substrate (data not shown). This might be related to C_2H_2 induced necrosis and wilting of plants like shown in Figure 7.

Fig. 8 Influence of C_2H_2 application on evapotranspiration from pots of *P. zonale* (flow-through system, 5 vol.% C_2H_2)



2.3.2.3 Availability of carbon in the substrate depending on C_2H_2 application

Table 2 shows the results of the carbon extraction from pots of *P. zonale* and *E. pulcherrima* with and without C_2H_2 treatment. In case of both plant species the carbon yield was the same with and without exposition to C_2H_2.

Tab. 2 Carbon extracted from sand planted with *P. zonale* and *E. pulcherrima* after 24h exposition to C_2H_2 (5 vol.%)

Treatment	Extracted C [µg / g root f.m.]	
	P. zonale	*E. pulcherrima*
- C_2H_2	914 ± 297	734 ± 246
+ C_2H_2	933 ± 154	852 ± 183

2.3.3 Closed (static) vs. aerated (dynamic) system

2.3.3.1 Denitrification measurement of planted and unplanted substrate

Figure 9 shows the (N_2+N_2O)-N emissions following irrigation of unplanted peat in a dynamic (flow-through-chamber) and a static (closed jar) experimental system. Emissions from the closed system proved to be significantly higher than those of the dynamic system and the difference between both systems increased with time. The standard deviation was remarkably low for denitrification measurement and never exceeded 19 % of the mean value. It was especially low in the static system. After 32 hours of incubation the experiment was interrupted as pots did not cease to emit N_2O and an end of the denitrification process was not foreseeable.

Similarly, it was observed that N emissions from planted pots were higher in the closed system compared to the dynamic system after 24 hours of incubation (Fig. 10). At first, emission rates were similar in both incubation systems. They increased steeply shortly after irrigation and reached a maximum after 12 hours. Then, there was a clear decline of emissions in the dynamic system while only a slight decrease was observed in the closed system. It has to be mentioned that after 24 hours of incubation the jar experiment had to be interrupted because fungal growth was

observed on plants. This indicated that the flow-through chamber setup was more suitable for denitrification measurement of planted substrate. However, emitted N_2O might be lost in this system by transport via the shoot to the atmosphere.

Fig. 9 Influence of incubation system, closed vs. flow-through chamber, on (N_2+N_2O)-N emissions from unplanted peat substrate following irrigation (5 vol.% C_2H_2)

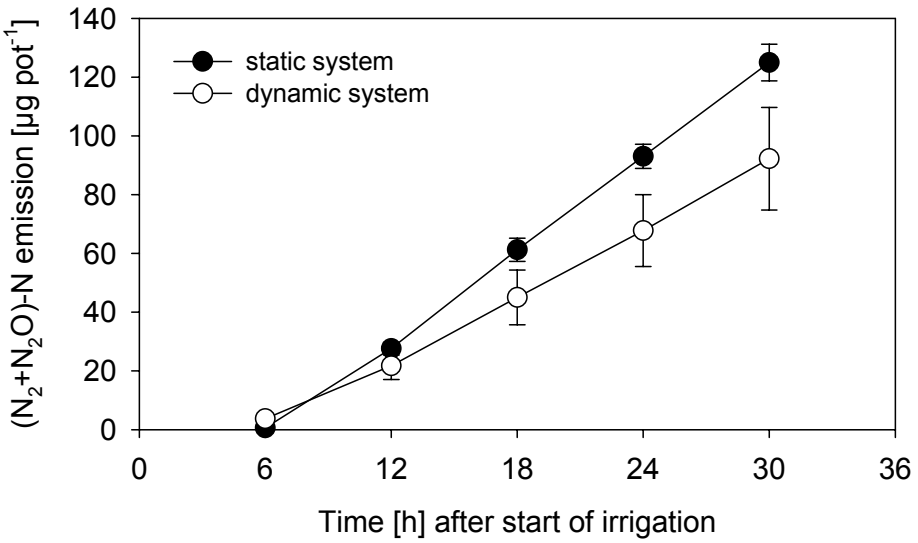
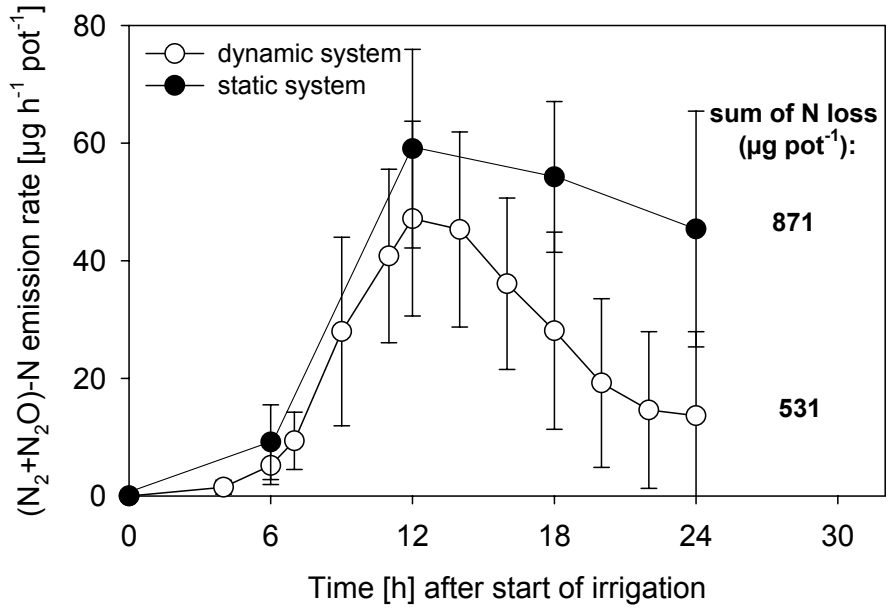


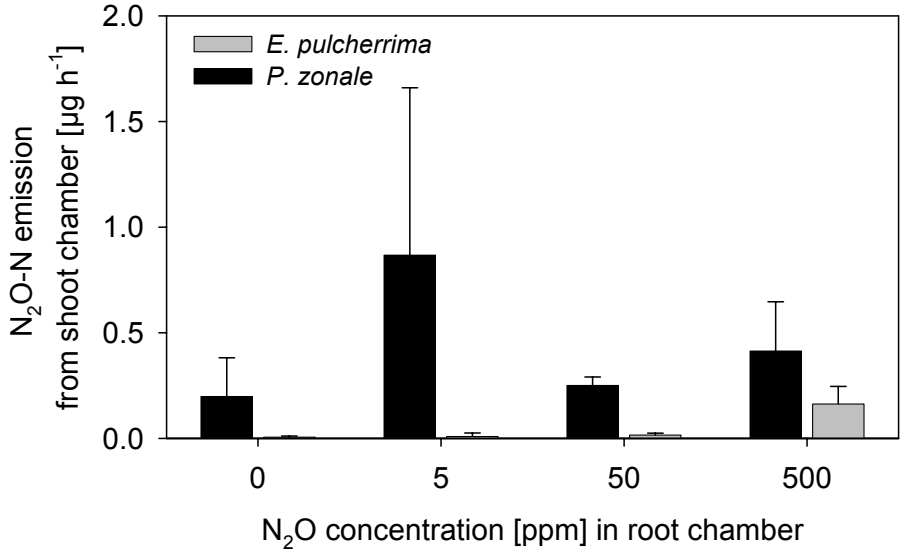
Fig. 10 Influence of incubation system, closed vs. flow-through chamber, on (N_2+N_2O)-N emission rates from pots of *P. zonale* in peat substrate following irrigation (5 vol.% C_2H_2)



2.3.3.2 Emission of N_2O by the plant shoot

During denitrification measurement of planted substrate in flow-through chambers the plant shoot remained outside the chambers. Transport of N_2O to the atmosphere by the shoot as observed with *Helianthus annus* (Ferch and Römheld 2001), *Brassica napus* and *Hordeum vulgare* (Chang et al. 1998) might lead to losses of N_2O and thus compromise the assay system. Consequently, experiments were conducted to investigate N_2O emissions from plant shoots of *P. zonale* and *E. pulcherrima*. N_2O was added to the root chambers to reach concentrations of 5, 50, and 500 vppm. As N_2O was on one hand consumed and on the other hand produced by the substrate, the intended values were not stable, but varied by about 30 % during incubation. All in all, only very low N_2O concentrations which hardly exceeded the value for ambient air could be measured from the shoot chambers. The calculated emission rates were accordingly low (Fig. 11). In case of *P. zonale* emission rates from the shoot chamber showed no relation to N_2O concentration in the root chamber. N_2O emission rates of *E. pulcherrima* were much lower than of *P. zonale* but they increased with N_2O concentration in the root chamber. As the measured N_2O values were close to the detection limit and negligible compared to N_2O production in the substrate, danger of falsifying denitrification measurement was estimated to be very low.

Fig. 11 N_2O-N emission from *P. zonale* and *E. pulcherrima* shoot depending on N_2O concentration in root chamber (incubation 25.5h)



2.4 Discussion

2.4.1 Application of acetylene (C_2H_2)

2.4.1.1 Optimum concentration of C_2H_2

The presented data show that a concentration of 1 vol.% C_2H_2 completely inhibited N_2O reduction (Fig. 4). This coincides with observations described in literature, where in lab experiments C_2H_2 was found to be an efficient inhibitor of N_2O reduction at very low concentrations of 0.1 vol.% or 1 vol.% (Yoshinari et al. 1977, Ryden et al. 1979a, Daum and Schenk 1997). Still, it was decided to use a higher concentration of about 5 vol.% for both, closed and dynamic system, in order to ensure constant inhibition of N_2O reduction since C_2H_2 concentrations fluctuated by $\pm$ 2 vol.% during measurement in flow-through chambers. This resulted from slight changes in the amount of C_2H_2 released from the gas flasks in the greenhouse which was probably due to changes in pressure induced by variation of temperature and solar radiation during day.

2.4.1.2 Optimum duration of C_2H_2 pretreatment

Depending on the duration of incubation, C_2H_2 pretreatment from 0 to 24 hours produced different effects on N emissions (Fig. 5). After 24 hours of incubation overall differences were small between treatments but still, statistically significant differences were found between the 0 and 2 hour pretreatments on one hand and the 12 and 24 hour pretreatments on the other hand. In contrast to this, after 48 hours of incubation there seemed to be a difference between unpretreated substrate and pretreated substrate independent of the length of preincubation.

The relatively low N loss observed after 24 hours of incubation in the 0 and 2 hour pretreatment might have resulted from insufficient distribution of C_2H_2 within the substrate and subsequent incomplete inhibition of N_2O reductase. Yet, pretreatment with C_2H_2 is also practised in field experiments and Ryden et al. (1979b) demonstrated that short periods of 15 to 30 minutes were sufficient for C_2H_2 diffusion into soil. Considering the high air filled pore space of peat relative to mineral soil it seems unlikely that two hours of pretreatment should not have been efficient in distributing C_2H_2 within the substrate. On the other hand, the reason for the observed

difference might not be due to suboptimal but to supra-optimal C_2H_2 application. In literature there are reports on promoting effects of C_2H_2 on denitrification. This was attributed to increased availability of carbon either from acetone, a contaminant of C_2H_2 gas (Gross and Bremner 1992) or from C_2H_2 decomposition (Payne 1984). In another case a promoting effect of C_2H_2 was observed but could not be related to carbon availability (Yeomans and Beauchamp 1982). Possibly, the 12 and 24 hour C_2H_2 pretreatments lead to a relative increase in carbon availability that resulted in slightly higher N emissions after 24 hours of incubation.

After 48 hours of incubation N emissions from the unpretreated substrate were lower than from pretreated substrates. This difference in N loss was statistically non-significant. Still, it indicated inferiority of incubation without pretreatment, possibly because of incomplete inhibition of N_2O reduction, and equality of the 2, 12, and 24 hour pretreatments with regard to N_2O yield. Conformity between the 2 and 12 hour pretreatment was confirmed in a flow-through chamber experiment with planted substrate (Tab. 1).

Consequently, it was decided to use C_2H_2 pretreatement for experiments. To reduce potential side effects of C_2H_2 a pretreatment period of 2 hours was chosen.

2.4.2 Side effects of C_2H_2

Application of C_2H_2 for more than 48 h lead to irreversible leaf damage on both, *P. zonale* and *E. pulcherrima* (Fig. 6, 7). *P. zonale* appeared to be the more sensitive species as it reacted sooner and more uniformly than *E. pulcherrima*. While *P. zonale* soon developed chlorosis during C_2H_2 application *E. pulcherrima* showed a slight leaf epinasty. Both symptoms are reported to be related to ethylene exposition (Abeles et al. 1992, Serek and Prabucki 1998, Serek and Reid 2000), and traces of ethylene were contained in the C_2H_2 gas that was available for denitrification experiments due to its production process (Linde, personal information). The ethylene content of this type of gas was reported to be 73 vppm (Hyman and Arp 1987), while the threshhold for physiological activity is reported to be about 0.01 vppm (Abeles et al. 1992). If plant reaction was caused by ethylene only or by acetylene which is an ethylene analogue is not known.

In contrast to obvious shoot damage, an effect of C_2H_2 treatment on plant roots characterised by evapotranspiration (Fig. 8) and carbon content of the substrate

(Tab. 2) could not be observed. This is in agreement with Daum and Schenk (1997) who reported a significant increase in O_2 consumption by roots of cucumber seedlings in nutrient solution only after four days of C_2H_2 treatment (10 vol.%). The denitrification event in planted peat needed less time and mainly took place during the first 24 hours of incubation. It was concluded that within the critical time period no increased O_2 consumption occurred in *P. zonale* and *E. pulcherrima*.

The results indicate that C_2H_2 should be applied only for short periods of one or two days to planted substrates to avoid irreversible damage of plants. Short-term application of C_2H_2 has been recommended often in literature, but with regard to incomplete blocking of N_2O reductase due to decomposition of C_2H_2 or adaptation of microorganisms to C_2H_2 (Watanabe and De Guzman 1980, Yeomans and Beauchamp 1982, Rolston 1986) or with regard to stimulation of denitrification (Topp and Germon 1986). These problems occurred only after applying C_2H_2 for periods that were longer than necessary for denitrification measurement in planted horticultural substrates.

2.4.3 Closed (static) vs. aerated (dynamic) system

From the closed system higher amounts of gaseous N were recovered than from the dynamic flow-through system after incubation of unplanted peat (Fig. 9). The reason for this might be technical, as the glass jars used for static incubation were perfectly air tight and allowed no loss of N gases. The flow-through chambers in contrast had openings which might have allowed diffusion of N gases inspite of the air stream. Also, precise airflow depended on perfect and stable functioning of pumps and airflow meters. Maybe diurnal changes in the performance of technical equiment lead to variability of airflow. It can only be speculated if this might have lead to underestimation of N loss, but it is likely that it contributed to the higher standard deviation observed in the flow-through system (Fig. 9). A decrease of substrate water content which might have accounted for lower N emissions in flow-through chambers during incubation was not observed.

While the closed system appeared to be superior for studying unplanted substrate, difficulties arose when planted substrate was incubated. Within 24 hours plants were infested with fungi which was probably due to high air humidity within the jars. Additionally, the course of N emissions was not the same in both systems. While

emissions from flow-through chambers decreased and finally ceased, they remained at a high level in closed jars (Fig. 10). The decline of N emissions in the flow-through system can be attributed to alleviation of oxygen deficiency within the substrate due to water uptake by the plant and subsequent diffusion of air into substrate pores (see Chapter 4). The lacking air exchange in the closed system inhibited transpiration, and thus hindered diffusion of air into the substrate. Therefore, closed jars lead to an overestimation of N emissions and were not suitable for incubation of planted substrate. Exclusion of the shoot from the closed compartment was not considered as it required openings in the jar which were likely to allow diffusion of accumulating N gases.

When N_2O emission from the shoots of *P. zonale* and *E. pulcherrima* plants was measured, only low values close to the detection limit of N_2O were obtained (Fig. 11). While emissions from *E. pulcherrima* increased with N_2O concentration in the root chamber, no such relationship could be observed with *P. zonale.* It was speculated if the observed N_2O concentrations in the shoot chamber might result from diffusion of gas from the root chamber because sealing of both, shoot and root compartment, proved to be difficult. On the other hand, both chambers were separated by spacers so that diffusing N_2O was more likely to spread in the room. Ferch and Römheld (2001) clearly documented transpiration driven N_2O transport from root to shoot of *Helianthus annuus*, and the same was reported for *Brassica napus* and *Hordeum vulgare* by Chang et al. (1998). With regard to denitrification measurement in flow-through chambers, release of N_2O from the plant shoot would lead to loss of N_2O from the assay system which excludes the plant shoot. In the presented study, it was not clear if the calculated emission rates truly represented N_2O transport by the plant shoot, but the amounts of N_2O measured in the shoot chambers were too small to compromise denitrification measurement in flow-through chambers. Like illustrated in Figure 10, N emission rates from peat substrate may reach 50 µg h^{-1} pot^{-1} whereas N emissions from the shoot hardly reached 1 µg h^{-1} pot^{-1}.

Thus, it was decided to use the dynamic flow-through system for investigation of denitrification from horticultural crops while the closed system was further used for incubation of unplanted substrate.

3. Factors controlling denitrification in unplanted peat substrate

3.1 Introduction

Denitrification causes loss of nitrogen from soils and leads to emission of environmentally harmful N_2O to the atmosphere. Data of N losses by denitrification published in literature were gathered, e.g., by Nieder et al. (1989) and von Rheinbaben (1990) and ranged from zero to about 200 kg N ha^{-1} $year^{-1}$ with highest emissions resulting from vegetable crops (Ryden and Lund 1980, Schloemer 1991). The contribution of N_2O-N emitted from agriculture to global efflux of N_2O to the atmosphere is estimated to amount to 40 % (Oenema et al. 2001). Consequently, denitrification in soils has been subject to thorough investigation by agricultural scientists for the past three decades. Only little attention has been paid to gaseous N losses from horticultural peat substrates, so it was the aim of this research to investigate denitrification N losses from substrates that are used for horticultural production of potted ornamental plants.

Several factors are essential for denitrification and for the share of N_2O among its end products. Denitrification requires oxygen deficiency and availability of nitrate and easily available carbon (Groffman 1991, Tiedje 1988, Rolston 1986). In natural soils these regulators of denitrification are influenced by a multitude of environmental factors (Tiedje 1988, Weiske et al. 1995). Oxygen availablity, e.g., depends on soil characteristics, climate (temperature, rainfall), cultivation system (irrigation) and metabolism of plant and soil microorganisms. Many factors interact and vary in time and space, causing extremely high variability of N emissions and making assessment of denitrification N losses difficult (Duxbury 1986, Tiedje et al. 1989).

Although undisturbed soil samples are considered essential for measurement of natural N emission rates (Tiedje et al. 1989), use of homogenized soil samples is recommended for basic research on denitrification (Duxbury 1986). Incubation under controlled conditions reduces variability resulting from the dynamic nature of denitrification, and also allows simultaneous incubation of a comparably large number of samples (Davidson et al. 1986).

Accordingly, it was decided to incubate homogenized samples under standard conditions for investigation of the main factors controlling denitrification in horticultural peat substrates. The focus of research was on those factors which proved essential in field and lab incubations of soil: oxygen and carbon availability, N supply, and temperature.

3.2 Materials and Methods

3.2.1 Experimental setup for denitrification measurement

All denitrification measurements were performed in closed systems and with unplanted peat substrate. Substrate was incubated in 250 mL glass bottles or in 1.5 L glass jars (Weck).

3.2.1.1 Glass bottle incubation

To investigate the influence of oxygen concentration on denitrification, 10 g of peat substrate were filled into glass bottles which were closed with silicon gaskets before incubation. Bottles were pretreated with C_2H_2 for measurement of (N_2+N_2O)-N emissions (Chapter 3.2.3). After pretreatment the atmosphere of the bottles was replaced by argon, argon/air mixtures or air as described in Chapter 3.2.2. Then, C_2H_2 was renewed only in bottles pretreated with C_2H_2 and incubation started.

3.2.1.2 Jar incubation

Except for the experiments mentioned above all measurements were carried out in 1.5 L glass jars as described in Chapter 2.2.1.
Before incubation substrate was weighed, evenly filled into 280 mL plastic pots and closed into jars. Then it was pretreated with acetylene (C_2H_2) (Chapter 3.2.3).
After pretreatment jars were opened and pots were flood irrigated within the jars with a nutrient solution. The composition of the solution differed according to treatment as described in Chapter 3.2.4.
After fertigation jars were closed, C_2H_2 was renewed and incubation started (growth chamber). Air samples were taken with syringes after 24 h and/or 48 h of incubation.

3.2.2 Incubation atmosphere

In all jar experiments incubation atmosphere consisted of ambient air. In bottle experiments substrate was incubated at varying oxygen concentrations. To adjust the atmosphere, all bottles were evacuated with a vaccum pump and subsequently flushed either with argon to remove oxygen or with air if oxygen was not to be reduced. Filling and evacuating cycles were repeated three times. Then, bottles were filled with 280 mL of argon, argon/air mixture or air according to their treatment.
In experiment 1, substrate was incubated either in argon atmosphere or ambient air.
In experiment 2 substrate was incubated in argon/air mixtures resulting in oxygen concentrations of 0, 1.13, 2.25, 4.5, 9. and 18 vol.%.

3.2.3 Application of acetylene (C_2H_2)

C_2H_2 inhibits the reduction of N_2O to N_2 (Yoshinari et al. 1977) and was used for determination of (N_2+N_2O)-N emission by denitrification. It was applied at concentrations of 5 or 10 vol.% during measurements. Immediate inhibition of N_2O reductase was obtained by pretreating substrates with C_2H_2 for two hours.

3.2.4 Substrates

For bottle incubation at reduced oxygen concentrations a commercial peat substrate (pH6, standard fertilization, salt content 1 g (L substrate)$^{-1}$) was used.
Two types of substrate were chosen for jar experiments: a sieved peat substrate (5 mm) that was used for cultivation of plants and contained decomposable organic matter (root residues) and a peat substrate amended with 1 mg L^{-1} dried and ground organic matter (*Lolium westerwoldicum*) to insure microbial activity. Both substrates were adjusted to pH6 and fertilized with a complete compound fertilizer unless their treatment required different fertilization (see Chapter 3.2.5). Only one of the two substrates was used per experiment.

3.2.5 Fertilization of substrate and composition of fertigation solution

The volume of fertigation solution applied was 100 mL or 150 mL per pot and depended on substrate moisture. Equal amounts of solution were applied within each experiment. The solution was taken up by the substrate by capillary rise until saturation. To avoid waterlogging, excessive water was removed from the jars after 2 hours of flooding.

For demonstration of the irrigation effect on denitrification substrate was either fertigated with 100 mL of a solution containing 200 mg KNO_3-N L^{-1} or it was not irrigated but mixed with 20 mg NO_3-N (KNO_3) before incubation.

Substrate amended with glucose received 100 mL per pot of a solution containing 0, 0.04, 0.16, 0.4, or 0.6 g glucose-C per litre. The glucose-C concentration per litre substrate thus corresponded to 0, 14, 71, 143, and 214 mg C.

The effect of increasing nitrate supply on denitrification was investigated by incubating a substrate fertilized with all nutrients but N. At fertigation pots received 150 ml of a solution containing 0, 50, 150, and 450 mg NO_3-N L^{-1}, respectively.

In the experiment with varying temperatures pots were fertigated with 150 mL of a solution containing 150 mg NO_3-N L^{-1}.

3.2.6 Incubation temperature

Bottles were incubated at 30°C in an oven, while jars were incubated in a growth chamber at 26°C. Only in the experiment dedicated to the effect of temperature substrate was incubated at 17.8, 26 and 30°C.

3.2.7 Analytical procedures

The analysis of N_2O in all air samples was performed by a gas chromatograph (Chrompack 9001) with an electron capture detector (ECD) according to a method described by Mosier and Mack (1980).

Mineral nitrogen was extracted by shaking 50 g of substrate with 200 mL 0.1M KCL for one hour. The NO_3- and NH_4-N content of the extraction solution was determined photometrically (Technikon Autoanalyzer).

3.2.8 Statistics

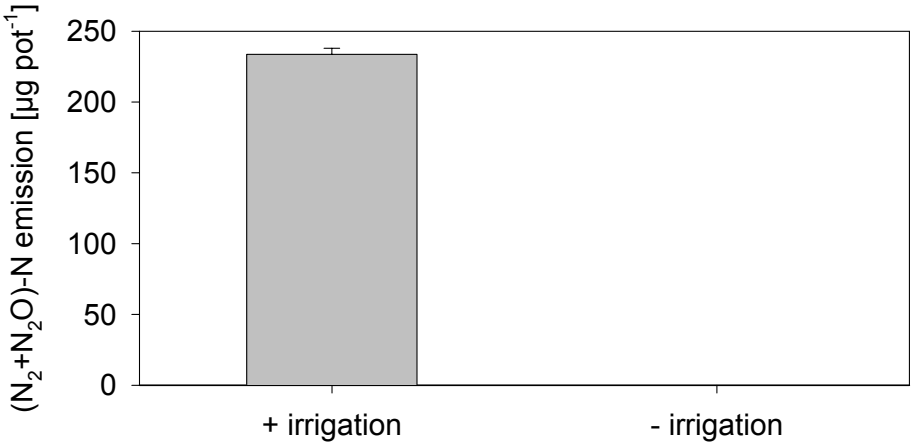
Statistic calculations were performed with the SAS package. The number of replications per treatment was three to five.

3.3 Results

3.3.1 Impact of oxygen availability on denitrification

Horticultural peat substrates of good quality characteristically maintain high air filled pore space during cultivation. Still, lack of oxygen may occur during irrigation, when air within soil pores is replaced by water. Thus, the effect of irrigation on gaseous N emission from a peat substrate was investigated (Fig. 1). While the flood-irrigated substrate produced high (N_2+N_2O)-N emissions within 24 hours the non-irrigated peat hardly produced any. The irrigation event seemed to be essential with regard to denitrification since it reduced oxygen availability in the substrate.

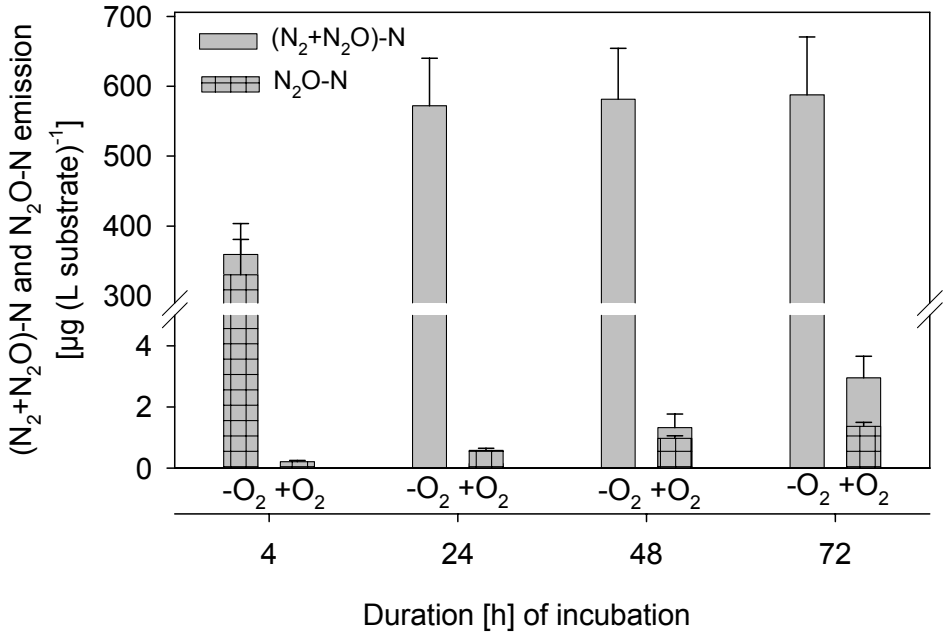
Fig. 1 Emission of (N_2+N_2O)-N from a sieved peat substrate depending on irrigation (incubation 24 h, 5 vol.% C_2H_2)



The importance of anaerobic conditions for denitrification was confirmed when substrate was incubated with and without oxygen (Fig. 2). Substrate incubated anaerobically (argon atmosphere) emitted tremendous amounts of (N_2+N_2O)-N while

under aerobic conditions only traces of gaseous N loss occurred. During anaerobic incubation high emissions of (N_2+N_2O)-N evolved from the substrate within the first four hours and increased only slightly during the following days. After four hours of incubation more than 90% of gaseous N loss consisted of N_2O-N whereas later N_2O could not be detected any more. Obviously it had been reduced to N_2 in the meantime.

Fig. 2 (N_2+N_2O)-N and N_2O-N emissions from peat substrate incubated aerobically (+O_2) and anaerobically (-O_2) (0 or 10 vol.% C_2H_2)



Another experiment was conducted to investigate the effect of oxygen concentration on denitrification (Table 1). It is obvious that only at 0 % oxygen there was an immediate production of N_2O. With rising oxygen concentration the time lag of N_2O emission increased. As the flasks were not opened until the end of incubation, oxygen content was likely to decrease because of consumption by microorganisms. It seemed that soil bacteria were in fact exhausting oxygen and that only then abundant production of N_2O was possible.

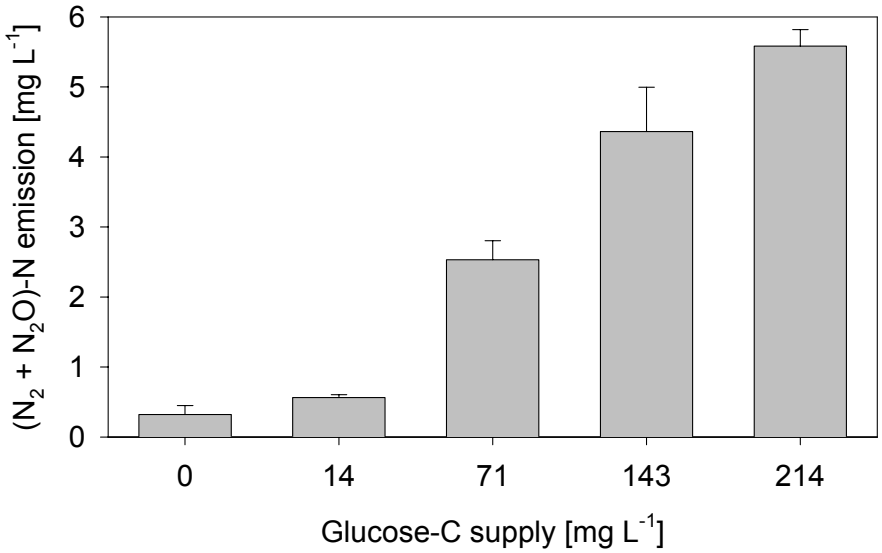
Tab. 1 (N_2+N_2O)-N emission from peat substrate at different concentrations of oxygen as affected by incubation time (10 vol.% C_2H_2)

O_2 concentration [%]	(N_2+N_2O)-N emission [µg (L substrate)$^{-1}$]		
	3.5 h	24 h	48 h
0	650.8	4607.0	4997.9
1.13	0.0	3030.7	3901.3
2.25	0.9	11.9	4325.8
4.5	0.0	0.2	24.6
9	0.0	0.5	7.3
18	0.6	0.3	2.9

3.3.2 Influence of carbon availability on denitrification

The effect of carbon application on denitrification N loss is shown in Figure 3. The peat substrate already contained organic material in form of root residues, so it produced noticeable amounts of N gases even without C fertilization. A clear rise in N gas production was observed with every increase in glucose supply up to the highest level, indicating that the potential of denitrification was enlarged by carbon supply.

Fig. 3 Influence of glucose-carbon supply on (N_2+N_2O)-N emission from peat substrate (24 h incubation, 10 vol.% C_2H_2)

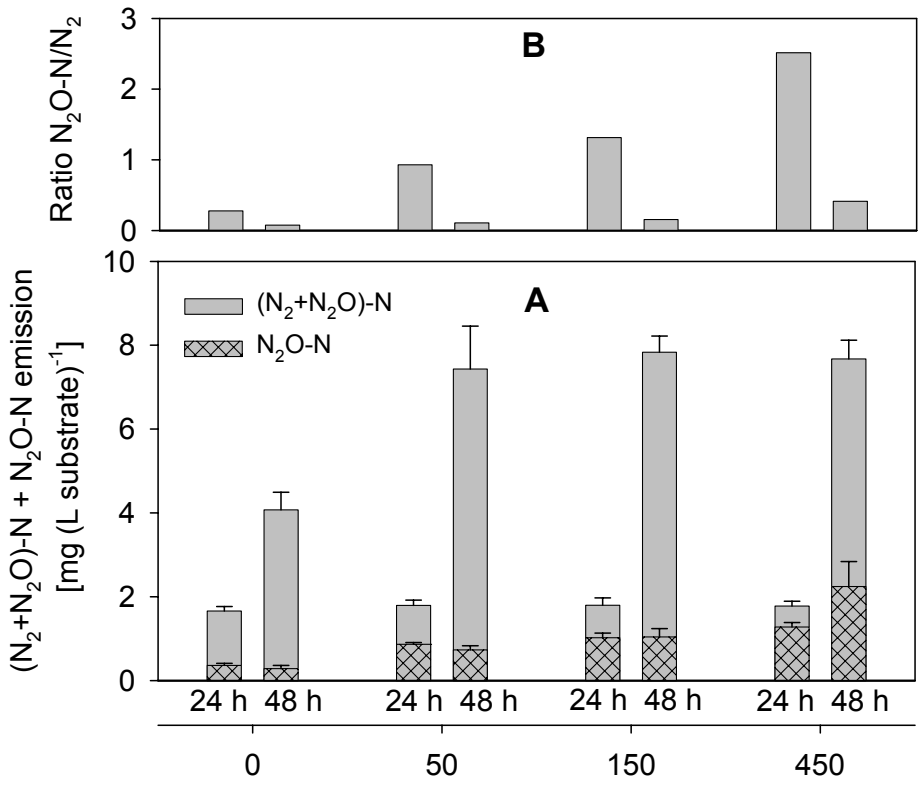


3.3.3 Effect of nitrate supply on denitrification

The effect of increasing nitrate supply on (N_2+N_2O)-N emissions from peat substrate was investigated (Fig. 4). Total N loss showed no significant differences between nitrate levels after 24 hours of incubation, but after 48 hours emissions from fertilized pots were significantly higher than from unfertilized pots. Regardless of the amount of NO_3 supplied, all fertilized pots produced nearly twice as much (N_2+N_2O)-N than unfertilized pots. In contrast, N_2O emissions increased steadily with NO_3 supply but were not affected by the duration of incubation. Only at the highest N level the N_2O content had approximately doubled.

The share of N_2O-N was high after 24 hours and increased with increasing NO_3 supply. At the highest nitrate level four times more N_2O-N than N_2-N was emitted. After 48 hours of incubation shares of N_2O-N were very small and even at the highest NO_3 treatment they only reached 50% of N_2 emission.

Fig. 4 Influence of nitrate-N supply on (N_2+N_2O)-N and N_2O-N emission from a peat substrate (A) and on the ratio N_2O-N/N_2 (B) (0 or 5 vol.% C_2H_2)



3.3.4 Influence of temperature on denitrification

The increase of temperature from 17.8°C to 30°C resulted in a steady rise in (N_2+N_2O)-N and N_2O-N emissions (Tab. 2). The Q10 value is the factor by which rate constants differ for a temperature interval of 10°C. For (N_2+N_2O)-N production it was 2.4 and 1.6 from 17.8°C to 26°C and from 26°C to 30°C, respectively. The share of N_2O-N increased with temperature.

Tab. 2 Effect of incubation temperature on (N_2+N_2O)-N and N_2O-N emissions from a peat substrate (24 h incubation, 0 % or 5 % C_2H_2)

	N emission [mg (L substrate)$^{-1}$]		
temperature [°C]	(N_2+N_2O)-N	N_2O-N	N_2O-N : N_2
17.8	0.46 a	0.17 a	0.59
26	1.10 b	0.47 b	0.73
30	1.80 c	0.83 c	0.85
	LSD = 0.11	LSD = 0.06	

* Different letters indicate statistically significant differences beween treatments (T-test, α = 0.05)

3.4 Discussion

3.4.1 Oxygen availability and source of N emissions

Anaerobic conditions were essential for production of N gases by denitrification (Fig. 2). Even low oxygen concentrations inhibited the denitrification process and reduced N emissions to zero during the first few hours of incubation (Tab. 1). Parkin and Tiedje (1984) and Firestone et al. (1979), too, reported drastic reductions of gaseous N loss by low oxygen concentrations of 3 vol.% and 1.7 vol.% relative to anaerobic incubation. Firestone et al. (1979) also observed a later increase in N loss inspite of low oxygen treatment and they pointed out that oxygen was consumed during incubation, thus inducing anaerobiosis. The same was assumed for the increasing N emissions during incubation of peat at low O_2 concentrations (Tab. 1).

During air incubation of peat substrate, very small but steady accumulations of N_2O-N and (N_2+N_2O)-N were observed (Fig. 2). The source of these emissions was not clear. Denitrification was considered improbable as the incubated substrate was very dry (~12 vol.% H_2O) which seemed to exclude existence of anaerobic zones. Sexstone et al. (1985b) investigated denitrification activity and oxygen profiles of soil aggregates and reported that denitrification was limited to aggregates with anaerobic zones. Still, there have been reports on aerobic denitrification (Robertson and Kuenen 1991) and many on N_2O production by nitrification (Yoshida and Alexander 1970, Bremner and Blackmer 1979, Bollmann and Conrad 1998, Davidson et al. 1986, Skiba et al. 1993, Koops et al. 1997). Robertson & Tiedje (1987) observed N_2O production from incubated forest soil which could not be attributed to denitrification nor to nitrification and they concluded that some other organisms, possibly fungi, might be its source. Whatever the cause, aerobic N emissions never exceeded 0.5 % of anaerobic N emissions (Fig. 2). Consequently they were considered insignificant and unlikely to compromise denitrification measurement in the presented system.
Emission of gaseous N (N_2+N_2O) from horticultural substrate proved to result mainly from denitrification and was dependend on oxygen deficiency. During horticultural plant production irrigation is the only factor which frequently induces anaerobiosis in substrates, so, like indicated by Figure 1, N emissions seemed to be exclusively linked to irrigation.

3.4.2 Influence of carbon availability on denitrification

Denitrification in peat substrate seemed to be severely limited by carbon supply (Fig. 3). The availability of carbon has often been referred to as most limiting factor of denitrification in soils (Swerts et al. 1996, Drury et al. 1991, Drury et al. 1998, Weier et al. 1993). However, anaerobic incubation of peat substrate that was not amended with carbon (Tab. 1) produced N losses that were comparable to those emitted after addition of 143 mg C L^{-1} during aerobic incubation (Fig. 3). The importance of oxygen deficiency over C availablility has been pointed out by Christensen et al. (1990a) who observed a higher intensity of denitrification after flooding than after addition of glucose. Similarly, Kapp et al. (1990) observed higher denitrification potential during incubation at optimum conditions compared to field incubation. They concluded, like Burford and Bremner (1975), that under anaerobic conditions denitrification was

controlled by C supply. Consistently, the presented data indicate that denitrification in peat substrate depended predominantly on anaerobiosis.

3.4.3 Effect of nitrate supply on denitrification

Nitrate supply only affected (N_2+N_2O)-N emissions at extremely low levels after 48 hours of incubation (Fig. 4). While N emissions were low when substrate was irrigated with water only, denitrification N loss was the same at all fertilization levels. This indicated that beyond a very low nitrate supply denitrification in peat substrate was not limited by nitrate, but by carbon. Ottow (1991) calculated exemplary that nitrate would only be the restricting factor if the ratio of NO_3^- to H^+ donator was 1:5 or even smaller. The equal N loss observed at all fertilized levels indicates that denitrification was independent of nitrate and that more NO_3^- than H^+ was available (Ottow 1991, Abou Seada and Ottow 1988). In literature, limitation of denitrification by NO_3 supply is considered to be restricted to nitrogen poor, natural ecosystems and was often not observed in arable soils (Myrold and Tiedje 1985, Ryden and Lund 1980, Drury et al. 1991, Weier et al. 1993).
In contrast, N_2O-N emissions increased with nitrate supply and consequently lead to rising N_2O-N:N_2 ratios. This increasing effect of nitrate availability on N_2O emissions was often described in literature (Ryden and Lund 1980, Sahrawat and Keeney 1986, Firestone et al. 1979, Weier et al. 1993) and attributed to "preferential acceptance of electrons by NO_3 over N_2O" (Swerts et al. 1996).
Nitrate supply in horticultural substrate is usually kept at a relatively high level by fertigation during cultivation. Thus, limitation of the denitrification process because of low nitrate availability is not to be expected in horticultural production. Increased nitrate fertilization is unlikely to promote denitrification in total, but it may lead to increased emission of environmentally harmful N_2O.

3.4.4 Influence of temperature on denitrification

Increasing temperature showed a promoting effect on denitrification (Tab. 2), which is consistent with observations reported in literature (Stanford et al. 1975, Blackmer et al. 1982, Ottow 1991). According to von Bischopinck and Ottow (1985) Q10 values for denitrification in soil incubated anaerobically were 1.6 to 1.9 for temperatures from

10°C to 60°C. Stanford et al. (1975) reported a Q10 value of about 2 from 15 to 35°C for waterlogged soils of different types. The observed Q10 values from aerobically incubated peat ranged from 1.6 to 2.4 for temperatures from 17.8°C to 30°C. These comparatively high values in the aerobic incubation system were probably due to the effect of temperature on oxygen availability and spreading of anaerobic zones. This is confirmed by de Klein and van Logtestijn (1996) who observed a much stronger temperature effect on the denitrification rate of non-irrigated than of irrigated soil. They concluded that oxygen availability and anaerobiosis were more affected by increasing temperature in the non-irrigated soil.

3.4.5 Summary

The lab incubation showed that oxygen and carbon availablility were the factors which mainly controlled denitrification in horticultural peat substrates. Rising temperature increased total N emissions while NO_3 availability only influenced total N loss at extremely low levels which are not likely to be found in fertilized substrates. The share of N_2O from total N emissions was increased by both, increasing nitrate supply and temperature. The transferability of the presented results to planted substrate and greenhouse conditions will be discussed in the following chapters.

4. Dynamics of denitrification in planted peat substrate

4.1 Introduction

Nitrate is lost from plant production systems mainly by two ways: by denitrification and by leaching. The physiology of denitrification has become well known through laboratory research but still, knowledge of environmental parameters does not allow reliable prediction of denitrification activity at field scale (Groffman 1991, Mosier et al. 1996). Although denitrification is mainly controlled by anaerobiosis and availability of nitrate and carbon, it is a very complex and dynamic process as it is affected by many interacting physical, chemical, and biological soil parameters (Groffman 1991, Tiedje et al. 1989). The sensitivity of the denitrification process to spatial or temporal changes in the soil environment causes the characteristically high variability of gaseous N emissions (Folorunso and Rolston 1984). Tracing back the causes of high variation in denitrification requires thorough analysis of the soil in question. Consequently, the transfer of knowledge on denitrifying activity from soil to soil or from soil to substrate is limited.

Most research on denitrification has been carried out with agricultural soils, but it is the objective of this study to investigate denitrification in horticultural substrates and cultures of potted ornamental plants. In previous studies of denitrification unplanted peat substrate showed much commonness with agricultural soils (see Chapter 3). But, so far the plant had been left out of the assay system, as it exerted a strong influence on denitrification in the horticultural growing system (see Chapter 2). In literature, various effects of plants on denitrification are described. Stimulation of denitrifying activity in planted soils has been related to oxygen consumption by roots and to rhizodeposition, which both increase anaerobiosis and availability of carbon to denitrifiers (Mahmood et al. 1997, Wollersheim et al. 1987, Trolldenier 1989, Smith and Tiedje 1979). Reducing effects of plants on denitrification, on the other hand, were attributed to plant nitrate uptake and resulting substrate deficiency for nitrate respiration (Ryden 1983, Mahmood et al. 1997, Smith and Tiedje 1979).

To clarify the influence of plants on denitrification in a horticultural growing system, denitrification was measured in planted peat substrate. The focus of research was

laid on plant and substrate characteristics and therefrom resulting sources of variability of denitrification N loss.

4.2 Materials and methods

4.2.1 Experimental setup for denitrification measurement

Flow-through chambers like described in Chapter 2.2.2 were used for measurement of denitrification from planted substrate.
Before the start of the experiment by flood irrigation, substrates were pretreated with acetylene (C_2H_2) within the chambers as described in Chapter 4.2.2.
The irrigation event was defined start of the experiment. Air samples were taken with syringes topped with gauge needles from silicon tubes at the air outlet of the chambers every two to four hours until N_2O emissions ceased.

4.2.2 Application of C_2H_2

For determination of (N_2+N_2O)-N emission by denitrification, 5 % C_2H_2 was added to the chamber atmosphere. To guarantee immediate inhibition of N_2O reduction to N_2 substrates were pretreated for two hours with 5 vol.% C_2H_2 prior to irrigation.

4.2.3. Duration of fertigation and composition of fertigation solution

For denitrification measurement, pots were fertigated with 1 L of a solution containing 150 mg NO_3-N L^{-1}. The irrigation solution was released by an opening at the bottom of the chambers after two hours.
To investigate the effect of permant flooding on denitrification the fertigation solution remained in the chambers throughout the whole experiment.
During cultivation plants were fertigated with 1g L^{-1} of a full compound fertilizer (Flory3, Euflor) according to horticultural practice.

4.2.4 Substrates

Peat based substrates were sieved (5 mm), limed (pH6) and mixed with 1 g L^{-1} of a full compound fertilizer (Flory3, Euflor) prior to planting. The peat content of the substrate was either 100 vol.% or 80 vol.%. Additional components were compost or cocopor.

4.2.5 Plant material

Experiments were conducted with plants of *Pelargonium zonale* 'Grand Prix' and *Euphorbia pulcherrima* 'Sonora Red'. Plants were propagated by cuttings and rooted in small peat nuggets (Jiffy7) for two weeks. Then plants were potted into 340 mL plastic pots and cultivated for at least four weeks to guarantee rooting of the substrate. For each experiment plants of the same species and set were used, i.e. plants were of the same age and of similar growth.

4.2.6 Inhibition of transpiration

To reduce transpiration plants were covered with cut open plastic bags that allowed air exchange but still, increased relative humidity beneath the plastic cover.

4.2.7. Analytical procedures

4.2.7.1 N_2O

The analysis of N_2O in all air samples was performed by a gas chromatograph (Chrompack 9001) with an electron capture detector (ECD) according to a method described by Mosier and Mack (1980).

4.2.7.2 Water content

During denitrification measurement every four to eight hours pots were taken from the chambers and weighed. At the end of the experiment plants were harvested, the

fresh weight of the shoot and of the rooted substrate was determined. Then, the substrate was dried at 105°C and weighed again. Water content (vol.%) was calculated from pot volume, fresh weight of shoot, dry weight of rooted substrate, and fresh weight during the experiment.
The water content of unplanted potted substrate was determined by weighing the substrate before and after drying at 105°C .

4.2.7.3 Air filled pore space

The air filled pore space was calculated by substracting the water content (vol.%) from the total pore space. The total pore space was determined by means of the 'Quick-Method' described by Wrede (2001).

4.2.7.4 Vapour pressure deficit (vpd)

Temperature and relative humidity were determined during denitrification measurements. Both values were used to calculate the vapour pressure deficit (Murray 1967, Malberg 2002):

$$vpd = e°(T) - e$$

$$e°(T) = 0.6108\exp [17.27*T/(T+237.1)]$$

$$e = e°(T) * rh/100$$

vpd = vapour pressure deficit [kPa]
e°(T) = saturation vapour pressure [kPa]
T = Temperature [°C]
e = vapour pressure [kPa]
rh = relative humidity [%]

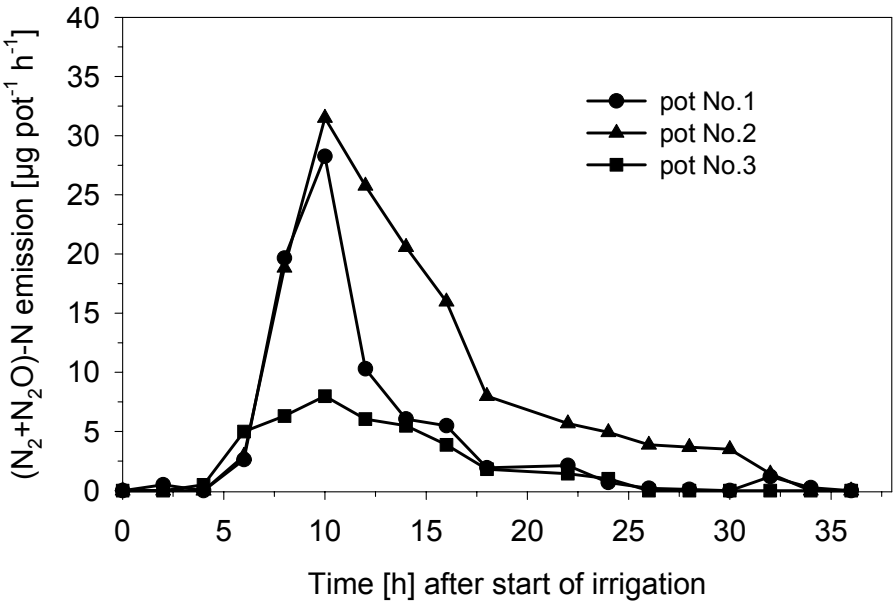
4.2.7 Statistics

Statistics were performed by use of the SAS package. Measurements were conducted with six replications per treatment.

4.3 Results

When denitrification N loss was measured in flow-through chambers from planted pots, N emissions always followed the same pattern (Fig. 1). Two to four hours after irrigation N_2O started to be emitted. Emissions increased more or less sharply until they reached a maximum and then decreased again towards zero. The end of emissions was often observed around 34 hours after irrigation. Although the pattern was the same, the height and also the width of the peaks differed amoung pots (Fig. 1). Thus, total N loss showed the characteristically high variability of denitrification measurements. In the following chapters causes of emission patterns and variability are investigated.

Fig. 1 (N_2+N_2O)-N emissions from a peat substrate planted with *P. zonale* following irrigation (5 vol.% C_2H_2)

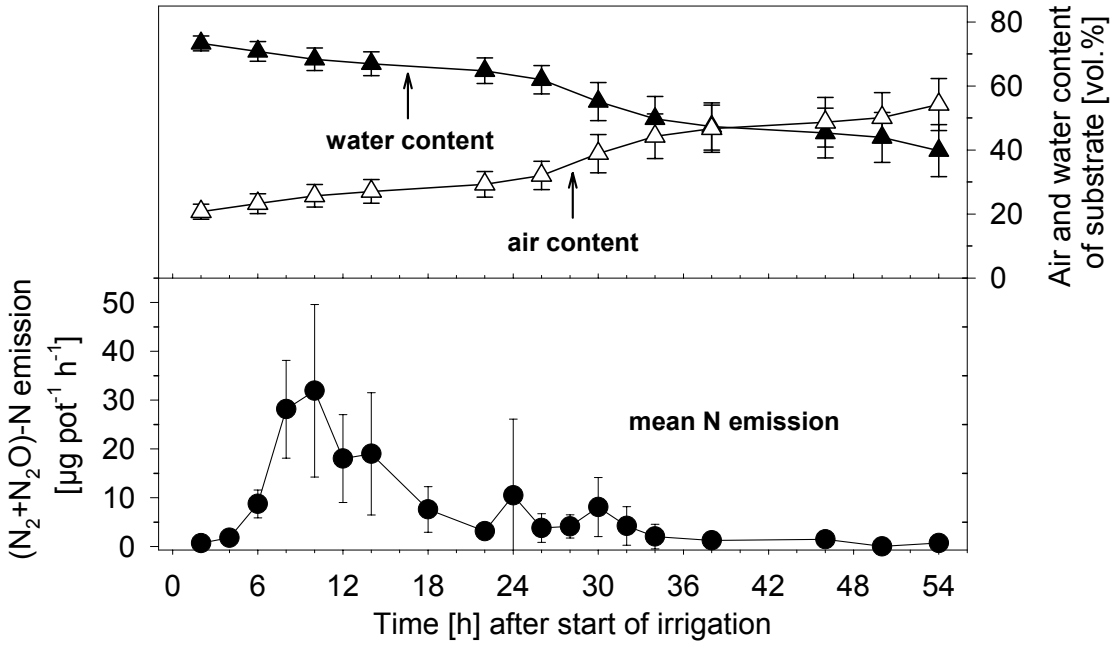


4.3.1 Influence of substrate air/water content on denitrification

In Figure 2 (N_2+N_2O)-N emission rates as well as air and water content of a peat substrate after irrigation are shown. Mean N emissions out of six replicates followed a peak shaped curve and showed high variability. Mean water content of the substrate decreased steadily from the end of irrigation on and with time its standard deviation

increased. As the air content was the reflection of the water content, it steadily rose after irrigation.

Fig. 2 (N_2+N_2O)-N emission rate, water and air content following irrigation of a peat substrate planted with *P. zonale* (5 vol.% C_2H_2)



When N emissions were related to substrate air content it resulted that below an average air content of 30 vol.% N was emitted at rates varying from 0 to 60 µg pot $^{-1}$ h^{-1} (Fig. 3). Yet, above 30 vol.% air no significant emissions occured at all. There seemed to be a threshold value above which denitrification was limited by presence of oxygen, which is also discussed in Chapter 6.

It was concluded that a decrease in N emission rate depended on a decrease of water content. To confirm this, N losses from planted pots were measured after 2 h and 52 h of irrigation (Fig. 4). After irrigation substrate water content of the control treatment steadily decreased, while that of the continuously irrigated substrate slightly increased. N emissions of both treatments did not differ much at first. But, after 22 hours emissions of the 2 h irrigated pots started to decrease while emissions of the permanently flooded pots further increased and never ceased until the end of the experiment. Thus, it seemed that the decrease of denitrification was primarily due to reduction of substrate water content and increased oxygen availability.

Fig. 3 (N_2+N_2O)-N emission rate depending on air filled pore space of a peat substrate planted with *P. zonale* (5 vol.% C_2H_2)

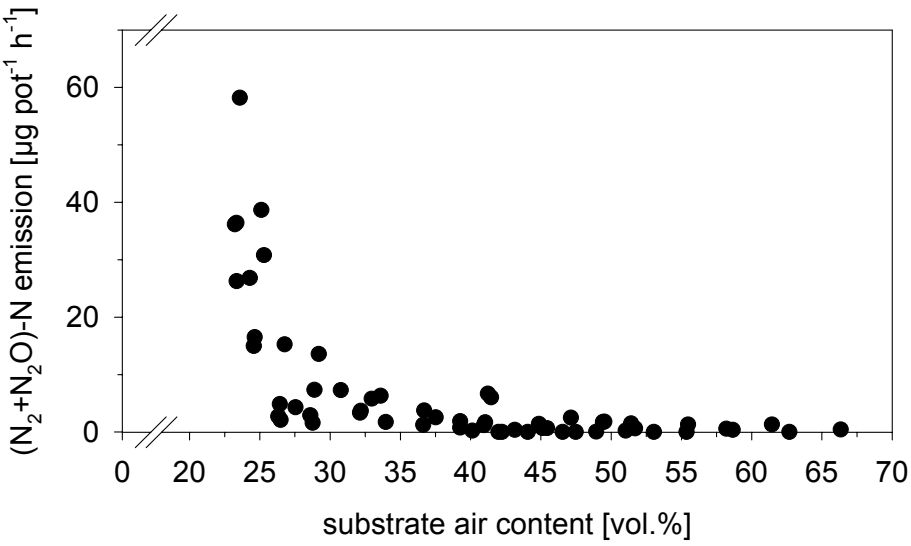
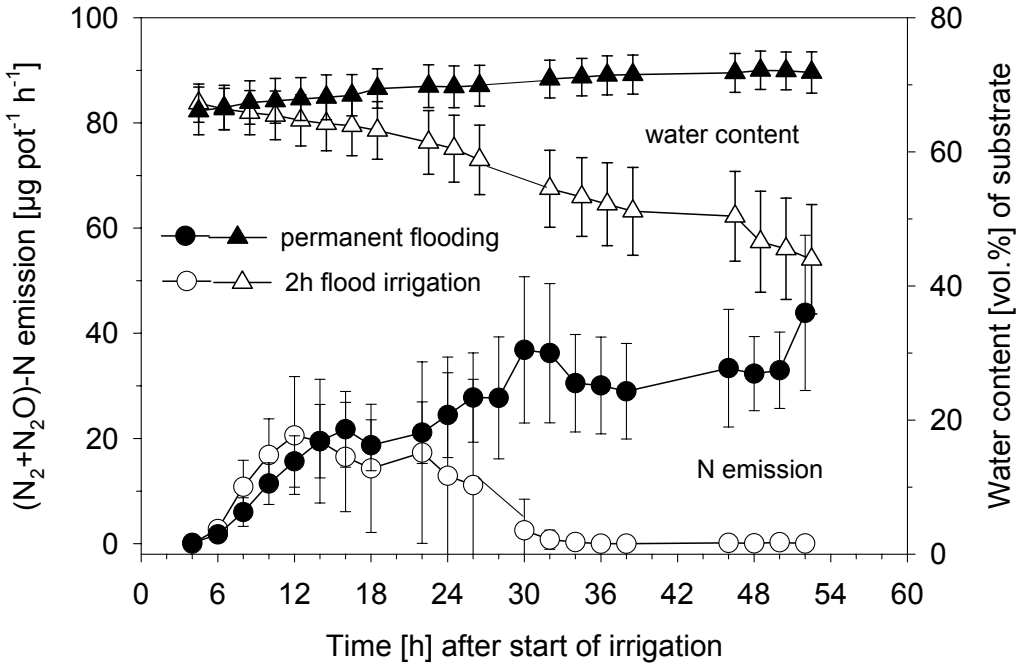


Fig. 4 (N_2+N_2O)-N emission rate and water content of peat substrate planted with *E. pulcherrima* following irrigation (2 h) or permanent flooding (5 vol.% C_2H_2)



4.3.2 Effect of transpiration on substrate air/water content and denitrification

Air and oxygen availability within a substrate depend on water content which is a dynamic parameter. The decrease of water content after irrigation is mainly due to uptake and transpiration by plants. Thus, the influence of transpiration on N emission rates and water content of planted substrates was investigated (Fig. 5). At first, N emission rates of both treatments, with and without inhibition of plant transpiration, were similar. But when emissions of the control treatment decreased, those of the inhibition treatment still rose and only sank when the transpiration blockage was relieved after 28 hours. The water contents of the substrates behaved accordingly. In the control treatment it decreased rapidly, while in the inhibition treatment it sank only slightly until the end of transpiration blockage.

In this experiment, transpiration proved to be an important factor influencing denitrification via water and air content of the substrate. The amount of water transpired by plants showed a close correlation to climatic factors, temperature and relative humidity, which are integrated in the vapour pressure deficit (vpd) (Fig. 6). The data was obtained during five denitrification measurements under different weather conditions and shows a strong increase of transpiration with increasing vpd.

Fig. 5 (N_2+N_2O)-N emission rate and water content of a peat substrate planted with *P. zonale* following irrigation with or without blockage of transpiration (5 vol.% C_2H_2)

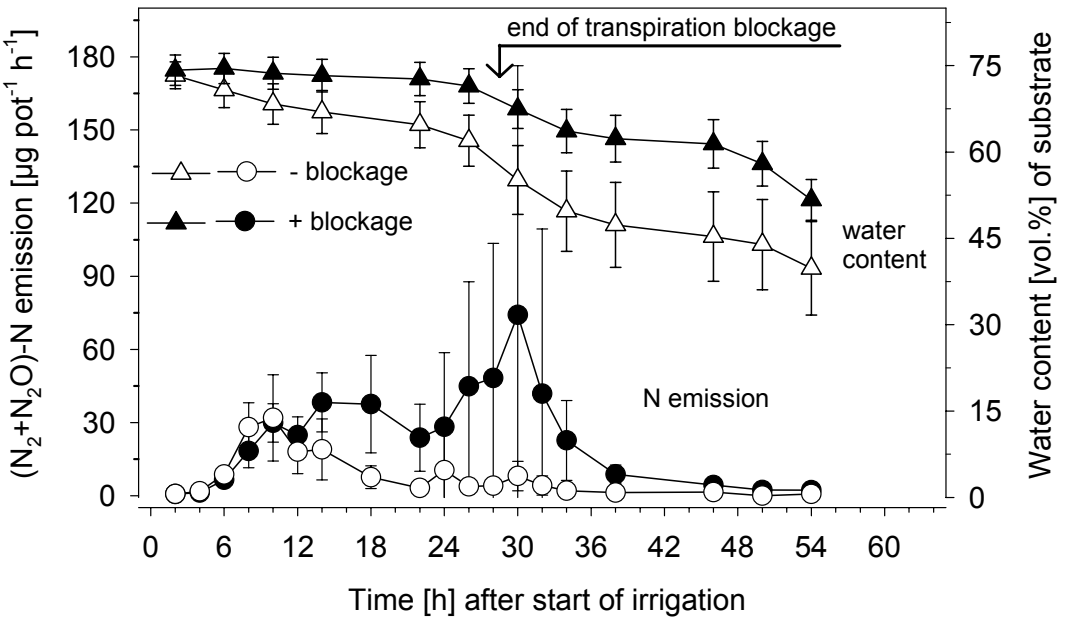
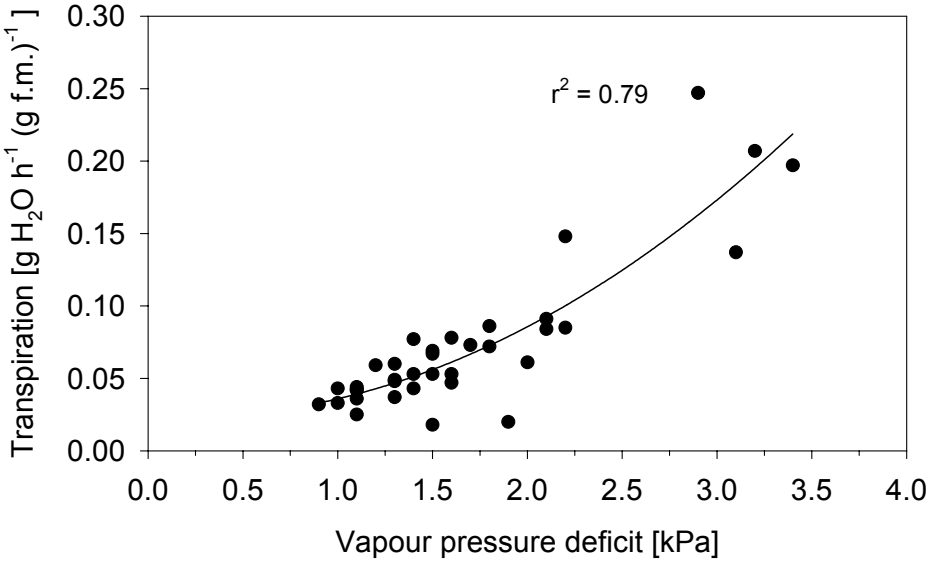
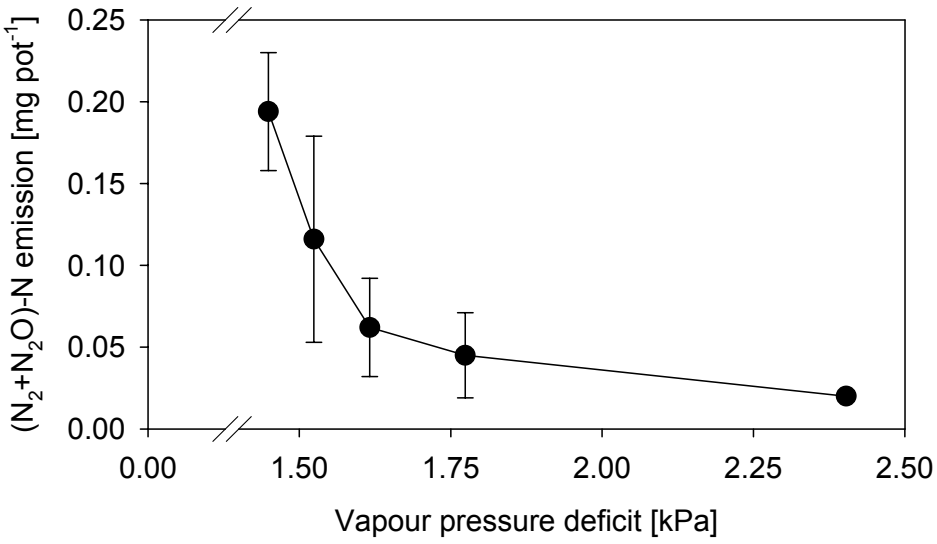


Fig. 6 Relationship of vapour pressure deficit and transpiration of *P. zonale* plants



When N loss of *P. zonale* pots from different measurements was related to vpd of the same dates, increases of vpd were indeed accompanied by decreases of N loss (Fig. 7). But still, there was some variation in N loss that did not correspond to vpd. Thus, it appeared that climate and transpiration were not the only factors controlling denitrification in planted substrates.

Fig. 7 (N_2+N_2O)-N emissions by pots of *P. zonale* planted in peat substrate and vapour pressure deficit

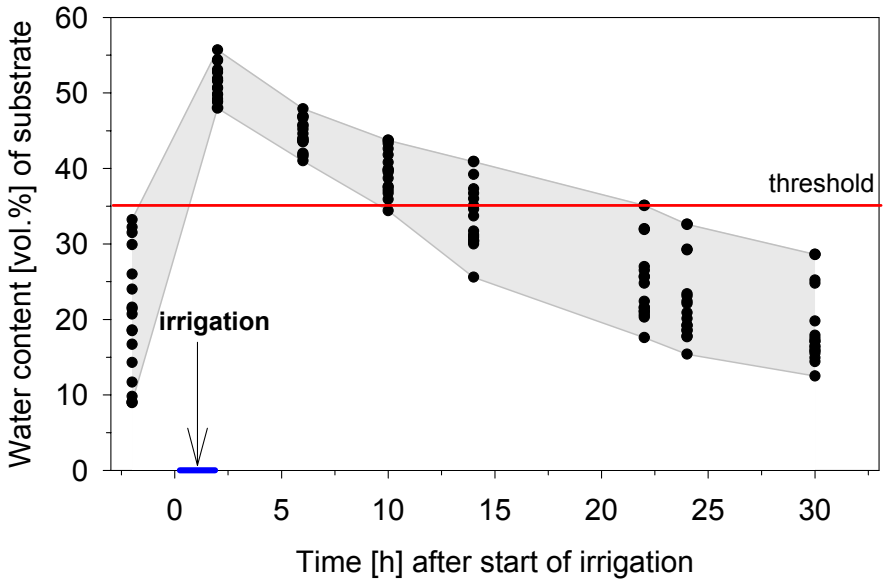


4.3.3 Influence of substrate moisture on air/water content after irrigation

During experiments it was observed that not only the drying of substrate after irrigation showed high variability, but that also the maximum water content that was reached after irrigation differed among pots (Fig. 8). The water content of pots varied strongly before irrigation and very dry substrates were included. After two hours of flooding water contents among pots still varied by up to 10 vol.%. With time variability grew stronger and often it were the same pots that maintained a very low or very high water content before and after irrigation. Consequently, the threshold value of water content for denitrification was reached after 10 hours or after 22.5 hours. This variability in duration of denitrifying activity was likely to be reflected in N loss. While variability of water content decrease after irrigation might be related to differences in plant size, the variability of maximum water content right after irrigation was probably due to substrate characteristics.

To investigate the relationship between water content before and after irrigation, potted substrate with adjusted water contents ranging from 10 to 70 vol.% were flood-irrigated (Fig. 9). Unplanted substrate was used to eliminate a possible influence of the plant. The positive relationship of water content before and after irrigation was very clear.

Fig. 8 Water content of a peat substrate planted with *P. zonale* before and after flood irrigation (2 h)



Yet, homogeneous water content and uniform distribution of water is unusual in planted substrate (Chapter 7). Still, when the experiment was repeated with planted substrate the positive relationship between water content before and after irrigation was the same as with unplanted substrate (Fig. 10).

Fig. 9 Relationship of water content before and after flood irrigation (2 h) of a peat based culture substrate

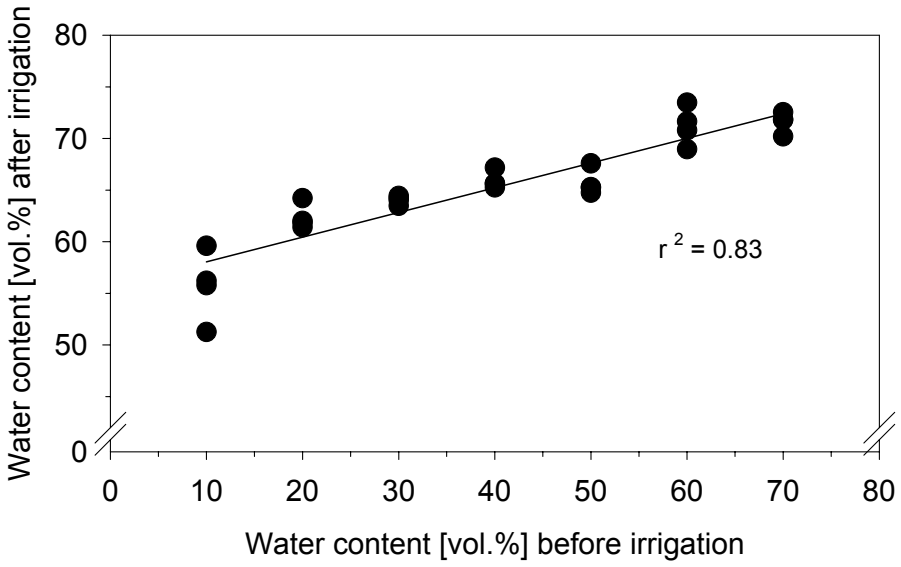
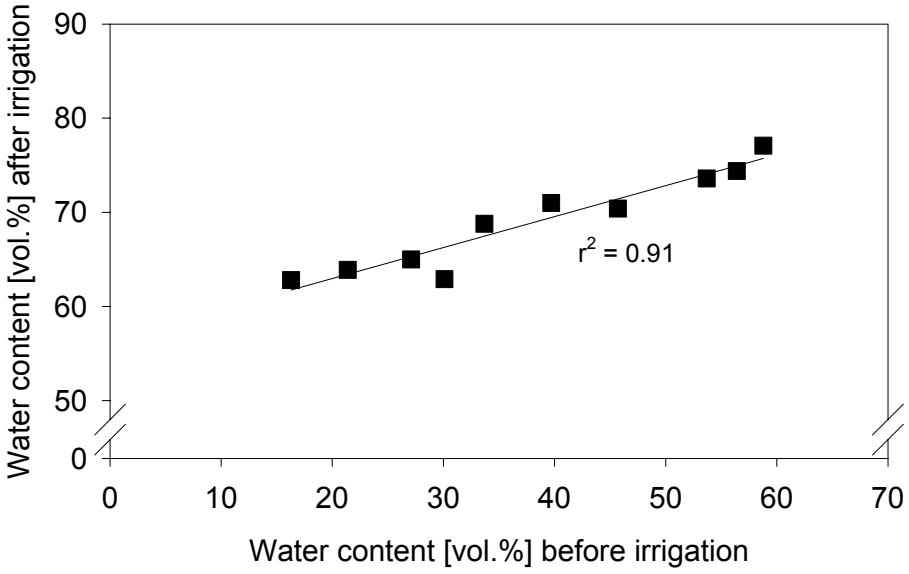


Fig. 10 Relationship of water content before and after flood irrigation (2 h) of a peat substrate planted with *P. zonale*



4.4 Discussion

4.4.1 Effect of substrate air and water content on denitrification

The flow-through system allowed to observe the dynamics of denitrification from undisturbed samples of planted substrate (Fig. 1, 2). N emissions were obviously related to the irrigation event as they increased significantly only a few hours after irrigation. The length or duration of the denitrification process seemed to be determined by oxygen availability. Right after irrigation the air filled pore space of the substrate was at its minimum, but it steadily increased during time following irrigation and N emissions were only observed as long as the average air content of the substrate was below a threshold value of 30 vol.% (Fig. 3). The dependency of denitrification on low air filled pore space was confirmed when return of air into the substrate was impeded by waterlogging (Fig. 4). Then, N emissions did not cease in contrast to emissions from the control treatment which decreased when substrate water content grew smaller after irrigation.

The above observations are in accordance with literature. Highest peaks of N emissions from soil were recorded after rainfall or irrigation events (Ryden and Lund 1980, Sexstone et al. 1985a, Bronson and Moiser 1993, Rolston et al. 1982) and denitrification N loss proved to be positively correlated with soil water content (Shelton et al. 2000, Weier et al. 1993, Linn and Doran 1984, Ryden 1983). Groffman and Tiedje (1988) used soil cores in a lab experiment to prove the promoting and inhibiting effect of wetting and drying cycles, respectively, on denitrification.

Even more than in experiments with unplanted substrate (Chapter 3), oxygen availability seemed to be the crucial factor for denitrification in planted peat substrate.

4.4.2 Threshold oxygen concentration

Data of N emissions and air content of the substrate allowed deduction of a threshold value for denitrification (Fig. 3). Above a value of approx. 30 vol.% air, which corresponds to 68 % water filled pore space (wfps), denitrification seemed to be limited by presence of oxygen and no more N emissions occurred. Still, the threshold was not uniform for all peat substrates and it showed considerable variation (Chapter 6). Also, it did not reflect air distribution within the substrate as it is only a mean value

of the substrate air content per pot. Consequently, deduction of the air content or oxygen level limiting denitrification at its very site of action was not possible.
The sudden rise of denitrification N emissions at a certain water level has often been observed and various threshold values for water content increasing or limiting denitrification are reported in literature. Values were often presented as wfps as this unit was considered to ease comparison between soils of different texture (Aulakh et al. 1991b, de Klein and van Logtestijn 1996). Still, values from literature vary strongly and range from 62 to 90 % wfps (Ryden 1983, Shelton et al. 2000, Aulakh et al. 1991b, de Klein and van Logtestijn 1996, Weier et al. 1993).
Thus, the observed value of 68 % wfps, or 30 vol.% air content of substrate, fits well into the reported range of threshold values and confirms the superior importance of the highly dynamic air and water balance of peat substrate with regard to denitrification. The cause of the variability of threshold values which has been observed in denitrification studies is further investigated in Chapter 6.

4.4.3 Influence of plant characteristics on denitrification

The dynamic nature of denitrification N emissions, i.e. increasing emissions after soil wetting and decreasing emissions during drying later on, became very clear in the presented study (Fig.1, 2, 4, 5). As discussed above, the course of N emissions was mainly influenced by dynamics of substrate water content. Transpiration and water uptake by the plant reduced substrate water content, which resulted in a termination of N emissions (Fig. 5). Evapotranspiration of each plant was influenced itself by climate and plant size (leaf area) (Fig. 6), which are both variable parameters. So, at least part of the observed variability in denitrification N loss was probably linked to variability of those factors that affect evapotranspiration.
The influence of the plant on denitrification presumably was especially high in this horticultural growing system, because plants only disposed of a very limited substrate volume (320 mL per plant). Within two to three weeks after planting of rooted cuttings the substrate volume was penetrated by roots and the degree of rooting further increased with time. Besides limiting denitrification by water uptake, plant roots likely promoted denitrification by consuming oxygen, especially when air exchange between substrate and atmosphere was inhibited by high water content (see also Chapter 5).

The promoting effect of plants on denitrification has been described in literature and was attributed to oxygen consumption and rhizodeposition (Smith and Tiedje 1979, Mahmood et al. 1997, Wollersheim et al. 1987, Prade and Trolldenier 1988). Limitation of denitrifying activity by plants was also observed, but it was ascribed to low nitrate availability in the rhizosphere due to nitrate uptake by plants (Ryden 1983, Smith and Tiedje 1979). There is only little information on the influence of plant water uptake on denitrification. Possibly this is because most research has been carried out in agricultural soils where under field conditions the influence of plants on soil water dynamics is less obvious than in the presented study.

4.4.4 Influence of substrate characteristics on denitrification

During denitrification experiments it was observed that the water content reached during flood irrigation varied between pots (Fig. 8). Still, the substrate volume per pot was the same, homogeneous substrates were used, and great care was taken at filling and planting of pots to avoid irregular compactions. Further investigations showed that a low water content before irrigation lead to a comparatively low water content after irrigation (Fig. 9, Fig. 10). It is likely that a low initial water content reduced the duration of denitrification, i.e. the time period until the threshold water content, below which denitrification was inhibited (Fig. 3), was reached (Fig. 8). Thus, inhomogeneity of substrate water content among pots was probable to be reflected in the variability of N loss.

The influence of substrate moisture on water uptake during irrigation is presumably related to the wettability of peat. It is known that many peat substrates become hydrophobic once they have been air-dried (Michel et al. 1999, Valat et al. 1991) and that drying reduces their water retention capacities. While it could be demonstrated that peat substrate with a lower water content before irrigation emitted less N during denitrification measurement (Chapter 6), drying of soil is reported in literature to promote denitrification. Groffman and Tiedje (1988) observed a stimulation of denitrification by drying and rewetting of soil, and they attributed this to increased aerobic soil respiration and thus reduced soil O_2 levels, and also to increased mineralization of C and N from dead microbial biomass. Patten et al. (1980) studied the effect of drying and air-dry storage of soils on the denitrification capacity. They incubated pretreated soils anaerobically and observed that drying increased the

denitrification capacity, which they ascribed to increased availability of soil organic matter. Studies by Lundquist et al. (1999) confirmed that sequential wetting and drying increased the amount of dissolved organic carbon in soil. As far as the author knows, there are no reports in literature on negative relationships between low soil water content before induction of denitrification and denitrification N loss. Yet, the problem of wettability is not restricted to peat, but has also been reported for soils (Wallis and Horne 1992). However, it might be more evident with peat because of its organic origin and its peculiar hydric behavior (Niggemann 1970, Valat et al. 1991).

5. Effect of plant age and carbon supply on denitrification

5.1 Introduction

Previous studies of denitrification from potted ornamental plants indicated that plants limited denitrification by decreasing substrate water content after irrigation and by thus relieving oxygen deficiency (Chapter 4). Yet, the influence of plant growth on the speed of water content decrease and on denitrification N loss during the growing period has not been investigated. Also, potted plants might have further effects on denitrification. While nitrate availability was considered granted by fertigation, it was estimated that carbon supply by plant roots may considerably increase denitrification. Studies with unplanted peat substrate indicated that carbon availability was a strong controller of denitrification in this growing medium (Chapter 3).

In literature, positive as well as negative plant effects on denitrification in soil have been reported. Smith and Tiedje (1979) measured increased denitrification rates in the rhizosphere of plants relative to the bulk soil. Yet, they pointed out that nitrate deficiency due to uptake by roots might still decrease denitrification. Similarly, Mahmood et al. (1997) reported higher denitrification from planted than from unplanted soil as long as nitrate availabilty was high. They attributed this to increased carbon availability. While planted and unplanted soil were often compared in literature, little information exists on the effect of increasing plant age or growth on denitrification and on the resulting variability of N loss during cultivation. Haider et al. (1987) carried out denitrification measurements during the growth cycle of maize. They observed a stimulation of denitrification by plants at the end of the growing period when root biomass declined. Quian et al. (1997) in contrast, observed higher N loss from maize planted soil at an early growth stage of plants. They attributed this to high root exudation of young plants and to high availability of nitrate in soil.

To clarify the influence of plant age on denitrification and to assess its contribution to variability of N loss during pot plant cultivation, experiments were conducted with four and eight week old plants of *P. zonale* as well as with and without glucose-C amendment of planted substrate.

5.2 Materials and methods

5.2.1 Experimental setup for denitrification measurement

Flow-through chambers like described in Chapter 2.2.2 were used for measurement of denitrification from planted substrate.
Before the start of the experiment by flood irrigation, substrates were pretreated with acetylene (C_2H_2) within the chambers as described in Chapter 5.2.2.
The irrigation event was defined start of the experiment. Air samples were taken with syringes topped with gauge needles from silicon tubes at the air outlet of the chambers every two to four hours until N_2O emissions ceased.

5.2.2 Application of C_2H_2

For determination of (N_2+N_2O)-N emission by denitrification, 5 vol.% C_2H_2 was added to the chamber atmosphere. To guarantee immediate inhibition of N_2O reduction to N_2 substrates were pretreated for two hours with 5 vol.% C_2H_2 prior to irrigation.

5.2.3. Duration of fertigation and composition of fertigation solution

For denitrification measurement, pots were fertigated with 1 L of a solution containing 150 mg NO_3-N L^{-1}. To investigate the effect of carbon availability on denitrification, 80 mg L^{-1} glucose-C were added to the nitrate solution. The irrigation solution was released by an opening at the bottom of the chambers after two hours.
During greenhouse cultivation plants potted in peat substrate were fertigated with 1g L^{-1} of a full compound fertilizer (Flory3, Euflor) according to horticultural practice.

5.2.4 Substrates and plant material

Substrates used for experiments were commercial, full fertilized white peat substrates.
Denitrification measurements were done with potted plants of *Pelargonium zonale* ‘Grand Prix’. Plants were propagated by cuttings and rooted in small peat nuggets

(Jiffy7) for two weeks. Then, plants were potted into 340 mL pots and cultivated for at least four weeks to guarantee rooting of the substrate.
Generally, plants of the same set were used, i.e. plants were of the same age and of similar growth. The effect of plant age was investigated on four and eight week old plants.

5.2.5 Analytical procedures

5.2.5.1 N_2O

The analysis of N_2O in all air samples was performed by a gas chromatograph (Chrompack 9001) with an electron capture detector (ECD) according to a method described by Mosier and Mack (1980).

5.2.5.2 Water content

During denitrification measurement every four to eight hours pots were taken from the chambers and weighed. At the end of the experiment plants were harvested, the fresh weight of the shoot and of the rooted substrate was determined. Then, the substrate was dried at 105°C and weighed again. Water content (vol.%) was calculated from pot volume, fresh weight of shoot, dry weight of rooted substrate, and fresh weight during the experiment.
The water content of unplanted potted substrate was determined by weighing the substrate before and after drying at 105°C .

5.2.5.4 Statistics

Statistics were performed by use of the SAS package. The number of replicates was six per treatment.

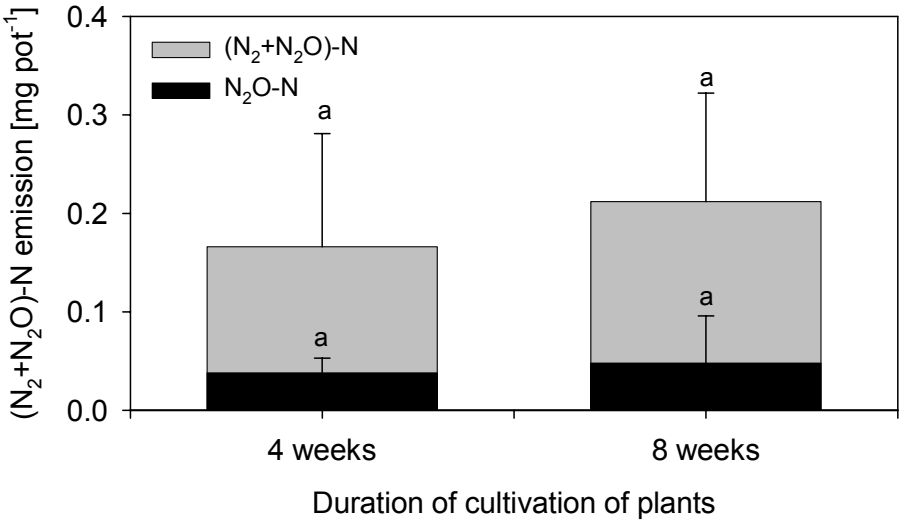
5.3 Results

5.3.1 Influence of plant age on denitrification N loss

N emissions from pots of four and eight week old plants of *P. zonale* were measured simultaneously to investigate the influence of plant age on denitrification. After four and eight weeks of cultivation plants had reached a mean shoot fresh weight of 13.8 g and 38.8 g, respectively. N loss tended to be higher from eight week old plants (Fig. 1). Yet, differences of mean emissions were non-significant.

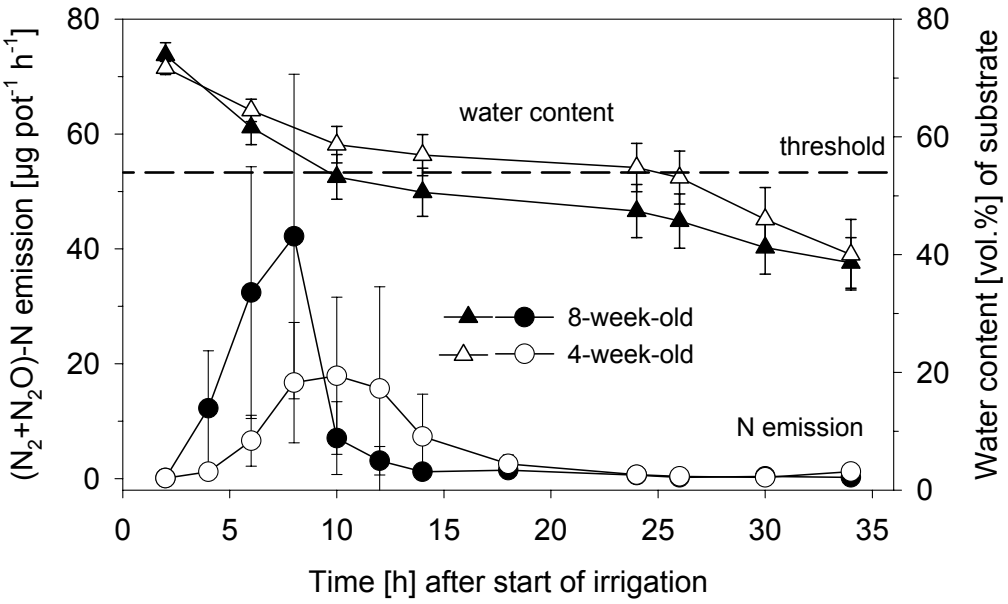
The course of N emissions, in contrast, showed noticeable differences (Fig. 2). Emissions from the older pots increased steeply after irrigation, reached a maximum after eight hours, and then declined just as steeply as they rose. No more N emissions could be found after 14 hours of incubation. The course of emissions from the four week old pots was more gentle, rise and decline were not as steep and the maximum emission rate was only half as high as of the older pots. But, emissions from the younger pots lasted for a longer period and ceased only 24 hours after irrigation.

Fig. 1 Influence of plant age on (N_2+N_2O)-N and N_2O-N emissions from potted *P. zonale* plants following irrigation (0 % and 5 vol.% C_2H_2)



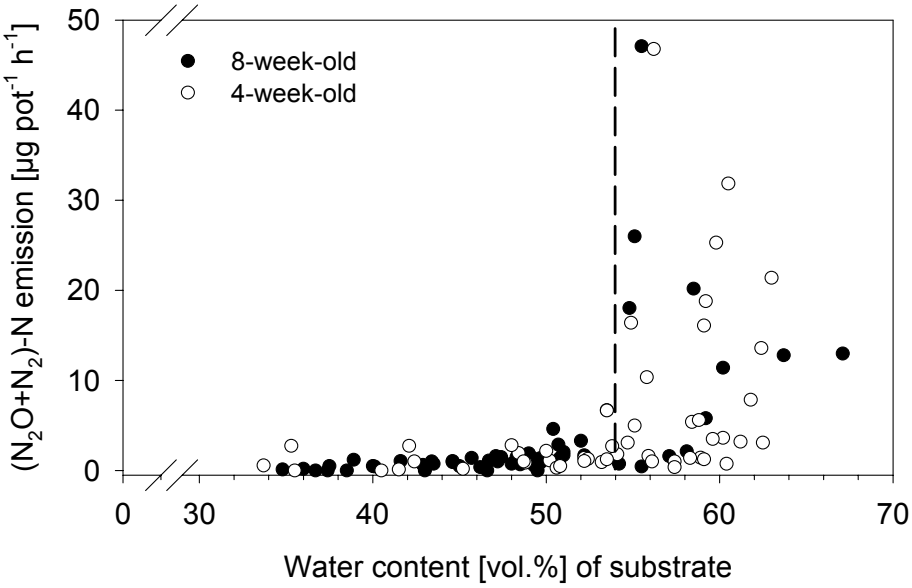
*Similar letters indicate statistically non-significant treatment effects (t-Test, α=0.05)

Fig. 2 Influence of plant age on (N_2+N_2O)-N emissions and substrate water content of potted *P. zonale* plants following irrigation (5 vol.% C_2H_2)



The decrease of substrate water content was more rapid in eight than in four week old pots (Fig. 2). When N emissions were related to substrate water content (Fig. 3), the same threshold value was observed for both treatments: gaseous N emissions only evolved at water contents above 54 vol.%.

Fig. 3 Influence of plant age on (N_2+N_2O)-N emissions and substrate water content of potted *P. zonale* plants following irrigation (5 vol.% C_2H_2)

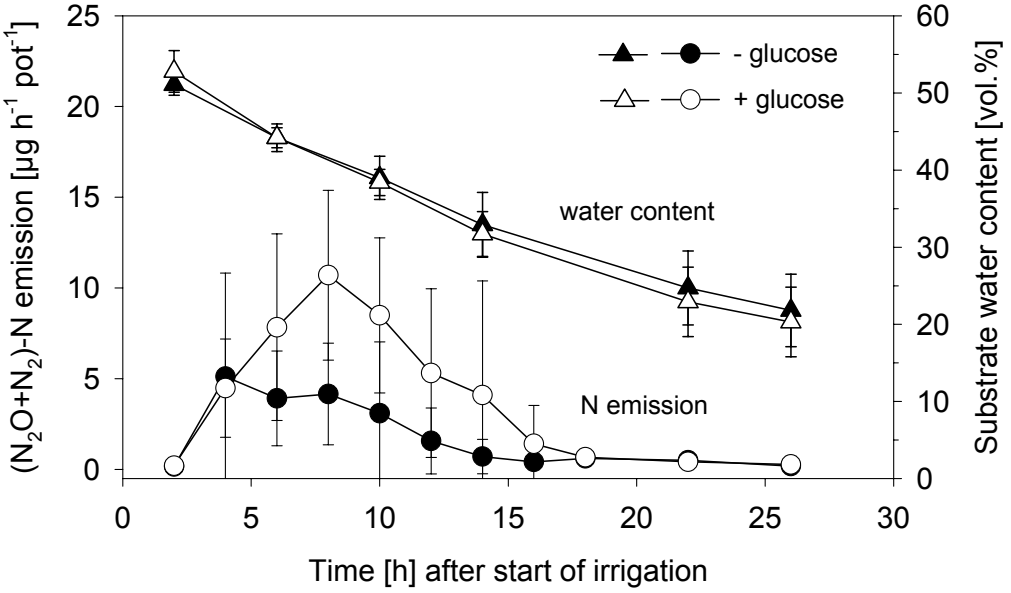


Although the duration of denitrifying activity seemed to shorten with increasing plant age, the older pots still tended to emit more N than the younger pots. It was speculated that higher emissions from older plants might have been due to increased carbon availability. Thus, the effect of carbon supply on denitrification in planted substrate was investigated in the next experiment.

5.3.2 Influence of carbon supply on denitrification N loss

When pots of *P. zonale* were amended with glucose-C, denitrification N loss was two times higher than from unamended control pots (Tab. 1). The duration of denitrifying activity was about the same in both treatments, but N emission rates were higher from glucose treated substrate than from untreated substrate (Fig. 4). These results indicated that denitrification in planted substrate was generally limited by availability of carbon.

Fig. 4 Influence of glucose supply on (N_2+N_2O)-N emissions from pots of *P. zonale* following irrigation (12 mg glucose-C per pot , 5 vol.% C_2H_2)



Tab. 1 Influence of glucose supply on denitrification N loss from pots of *P. zonale* following irrigation (12 mg glucose-C per pot , 5 vol.% C_2H_2)

treatment	(N_2+N_2O)-N [µg pot^{-1}]
+ glucose	87 ± 29 a*
- glucose	42 ± 23 b*

*Differing letters indicate statistically significant treatment effects (t-Test, α=0.05)

5.4 Discussion

N emission rates of eight week old pots increased sooner and reached a more than two times higher maximum than those of four week old pots (Fig. 2). The older plants had a nearly threefold higher shoot weight than the younger plants (Chapter 5.3.1), and it may be assumed that root mass also increased with plant age. Higher root mass might have lead to accelerated consumption of oxygen within the substrate and thus, to a faster increase of N emissions. Additionally, higher root mass might also have caused increased availability of carbon.

The stimulating effect of glucose-C supply on N emissions (Fig. 4) indicated that in planted peat substrate denitrification was limited by carbon availability. The increase of N emissions due to plant age (Fig. 2) was similar to that after glucose amendment (Fig. 4). Thus, an increase of carbon availability possibly had contributed to higher N emission rates from older relative to younger plants. Yet, with and without glucose amendment the rise of N emissions began at the same time, while N emissions increased sooner from pots of eight than of four week old plants (Fig. 2). This might hint at accelerated oxygen consumption by older relative to younger plants. Still, the threshold value of substrate water content was the same independent of plant age (Fig. 3).

In literature, there is clear evidence that denitrification in soil is promoted by low oxygen and high carbon availability (Parkin and Tiedje 1984, Weier et al. 1993). There is also evidence that plants may stimulate denitrification by increasing availability of carbon by rhizodeposition and by contributing to oxygen depletetion (Prade and Trolldenier 1988, Bakken 1988, Smith and Tiedje 1979). But, very little

information exists on plant effects on denitrification depending on age. Wollersheim et al. (1987) compared 7 and 14 day old plants and found no increase of N loss with plant age unless soil was compacted. Yet, the little difference in plant age might have reduced effects on denitrification. In other studies, denitrification was measured continuously during plant cultivation. But, these may not allow deduction of plant age effects because conditions for denitrification may change with time. Studies of Qian et al. (1997) and von Rheinbaben and Trolldenier (1984), e.g., indicated that changes of N emissions during plant growth might be due to changes in soil nitrate availability. Consequently, simultaneous measurement and uniform conditions for denitrification like in the presented study were considered necessary for evaluating the influence of plant age on denitrification.

From the presented results it may be concluded that stimulating plant effects by *P. zonale* on denitrification increased with increasing plant age. Own attemps to quantify C availability depending on plant age have failed (data not shown). Thus, it can only be speculated that both, increased carbon availability and oxygen consumption may have contributed to higher N emission rates from pots of eight relative to four week old *P. zonale* plants.

While eight week old plants produced higher N emission rates, the duration of denitrifying activity was about 10 hours shorter than that of four week old plants (Fig. 2). Bigger plant size and leaf area presumably lead to higher transpiration and thus, to the observed faster decrease of substrate water content in the older pots.

In literature, the most often encountered negative plant effect on denitrification is nitrate consumption. As far as the author knows no studies have focused on water content reduction as affected by plant age and its effect on denitrification. In the presented cultivation system the substrate volume per plant was comparatively low (320 mL) and thus, plant effects on water content were presumably much more pronounced than in natural soils.

This study confirmed, that plants exert positive as well as negative effects on denitrification, and it showed that these effects may change during plant growth. In the presented cultivation system, stimulating (oxygen consumption, C supply) as well as restricting effects (water consumption) on denitrification increased with plant age. The difference of summed up N loss between four and eight week old plants was small and statistically non-significant (Fig. 1). Thus, the contribution of plant age on variability of N emissions during cultivation of potted plants was considered to be low.

6. Physical substrate characteristics and denitrification

6.1 Introduction

N loss by denitrification is a factor which is often missing in N balances due to its difficult assessment. Apart from the requirement of technical equipment and elaboration of methodical practice, denitrification is typically subjected to high variability. This variability results from a multitude of variable, environmental factors which have an impact on the main controllers of the denitrification process, i.e. oxygen deficiency, availability of NO_3 and carbon, temperature and soil pH. Benckiser (1994) elaborated a very descriptive and complex scheme presenting the many factors and interactions which affect denitrification in soil. Beside chemical soil characteristics, like content of mineral nutrients, organic matter and pH, also physical soil properties have shown impact on denitrification, mostly by influencing water and air balance of a soil. For example, increased bulk density or soil compaction was reported to enhance denitrification in agricultural soils (Torbert and Wood 1992, Ruser et al. 1998, Bakken et al. 1987). Soil texture was suggested to affect denitrification by determining water holding capacity and air filled pore space (Aulakh et al. 1991b, Sexstone et al. 1985b).

These physical properties are also variable in horticultural substrates. The compaction of substrate is considered to be rather a product of potting practice of horticulturists, but there are more factors influencing physical substrate properties. Although they are mostly based on white peat, substrates vary in composition and quality. Often organic components like wood fibres and, to a lesser extent, rice husks are added to improve aeration of peat substrates. Also among peats there are differences in physical properties, depending e.g. on degree of decomposition, peat origin, and particle size (Uosukainen and Lötjönen 1997, Michiels et al. 1993). Especially the particle size has been repeatedly related to air and water balance of substrates (Scharpf 1997, Limbers and Rehme 1997, van Schie 1999, Verhagen 1997). In previous experiments, it was observed that water content of substrates after irrigation was not uniform, but depended on substrate water content before irrigation.

This was attributed to the problem of wettability of dried peat (Niggemann 1970, Valat et al. 1991), which apparently had compromised water capacity (Chapter 4).
As denitrification in horticultural peat substrates proved to be primarily controlled by water and air dynamics (Chapter 4), it was assumed that changes in physical substrate characteristics were reflected in denitrification N loss. To investigate the influence of physical substrate properties on denitrification, denitrification N loss was measured from planted substrate in flow-through chambers (Chapter 2). Additionally, changes in physical substrate properties which are most closely related to denitrification (pore space, air and water capacity), were surveyed by laboratory analysis ('Quick'-method) of unplanted substrate (Wrede 2001) .
The substrate treatments that were chosen for experiments were the following:

1. Drying of substrate (reduction of wettability),
2. Compaction of substrate (increased bulk density),
3. Sieving of substrate (reduction / homogenization of particle size) and
4. Substrate composition (addition of wood fibres or rice hulls to peat).

Additionally, pore volume, air and water capacity of planted and unplanted substrates were analyzed to confirm transferability of laboratory results from unplanted substrate to planted substrate of denitrification measurement.

6.2 Materials and methods

6.2.1 Determination of pore volume, water capacity, and air capacity

The 'Quick'-method by Wrede (2001) was used to determine total pore volume, water capacity, and air capacity of substrates. Only a short description of the method is given here, for more detailed information it is referred to the original paper.
The substrate to be analyzed was filled into test cylinders according to its volume weight (or in a higher density if required by the experimental aim). The volume weight was determined according to a standard method of VDLUFA (1991). Then, the cylinders were put into a basin filled with water and the substrate was left to saturate for 16 hours. After this, the cylinders were placed onto a water saturated layer of

sand contained in a riddled box. When water logging was removed from the sand, it exerted negative pressure on the substrate. Sand and substrate were left to equilibrate for eight hours. Then, shrinkage of the substrate volume and substrate water content were determined.
The following formulas were used to calculate pore volume, air capacity and water capacity:

Pore volume [vol.%] = (1- dB_E [g cm^{-3}] / dF [g cm^{-3}])*100
Water content [vol.%] = water content [weight %] * dB_E [g cm^{-3}]
Air content [vol.%] = pore volume [vol.%] - water content [vol.%]

dB_E = substrate density at the end of the experiment (considering shrinkage)
dF = density of the substrate dry matter (includes determination of organic and mineral substrate components)

$$dF\ [g\ cm^{-3}] = 100 / \left(\frac{\text{organic components [\%]}}{1.65\ [g\ cm^{-3}]} + \frac{\text{mineral components [\%]}}{2.65\ [g\ cm^{-3}]}\right)$$

To investigate the effect of plant roots on substrate properties, modified test cylinders of 10 cm diameter (785 mL) were used instead of the recommended cylinders of 7 cm diameter.

6.2.2 Denitrification measurement

6.2.2.1 Experimental setup

Flow-through chambers like described in Chapter 2.2.2 were used for measurement of denitrification from planted substrate.
Before the start of the experiment substrates were pretreated with acetylene (C_2H_2) within the chambers as described in Chapter 6.2.2.2.
After pretreatment with C_2H_2 pots were flood irrigated within the chambers.
The irrigation event was defined start of the experiment. Air samples were taken with syringes topped with gauge needles from silicon tubes at the air outlet of the chambers every two to four hours until N_2O emissions ceased.

6.2.2.2 Application of C_2H_2

For determination of (N_2+N_2O)-N production by denitrification, 5 vol.% C_2H_2 was added to the chamber atmosphere. For immediate inhibition of N_2O reduction to N_2 substrates were pretreated for two hours with 5 vol.% C_2H_2 prior to irrigation.

6.2.2.3. Duration of fertigation and composition of fertigation solution

Pots were fertigated with 1 L of a solution containing 150 mg NO_3-N L^{-1}. The irrigation solution was released by an opening at the bottom of the chambers after two hours of flooding.
During cultivation plants were fertigated with 1 g L^{-1} of a full compound fertilizer (Flory3, Euflor) according to horticultural practice.

6.2.2.4 Substrates and plant material

Substrates used for experiments were commercial, full fertilized white peat substrates with and without cocopor.
Additionally, some substrates were self prepared. Sieved (5 mm) or unsieved white peat substrates were limed (pH6) and mixed with a full compound fertilizer prior to planting. To investigate the effect of substrate composition on denitrification and substrate properties, mixtures of 70 vol.% sieved white peat and 30 vol.% rice husks, and 70 vol.% sieved white peat and 30 vol.% wood fibres were prepared. The substrates were adjusted to pH6 and fertilized with 1g L^{-1} of a full compound fertilizer.
Denitrification measurements were done with plants of *Pelargonium zonale* ‘Grand Prix’ and *Euphorbia pulcherrima* ‘Sonora Red’. Plants were propagated by cuttings and rooted in small peat nuggets (Jiffy7) for two weeks. Then, plants were potted into 340 mL plastic pots and cultivated for at least four weeks to guarantee rooting of the substrate.

6.2.2.5 Analytical procedures

6.2.2.5.1 N_2O

The analysis of N_2O in all air samples was performed by a gas chromatograph (Chrompack 9001) with an electron capture detector (ECD) according to a method described by Mosier and Mack (1980).

6.2.2.5.2 Water content

During denitrification measurement every four to eight hours pots were taken from the chambers and weighed. At the end of the experiment plants were harvested, the fresh weight of the shoot and of the rooted substrate was determined. Then, the substrate was dried at 105°C and weighed again. Water content (vol.%) was calculated from pot volume, fresh weight of shoot, dry weight of rooted substrate, and fresh weight during the experiment.

For determination of water distribution within potted substrate, peat was filled into 340 mL plastic pots. Pots were flood irrigated for two hours, then the substrate was divided into three horizontal layer starting from the pot bottom (0-2 cm, 2-4 cm, 4-7 cm layer). After drying at 105°C the water content of the substrate layers was calculated from fresh weight, dry weight, and volume of layer.

6.2.2.5.3 Statistics

Statistical analyses were conducted using the SAS software package. Three to six and six replications per treatment were used for measurements of physical substrate properties and denitrification, respectively.

6.3 Results

6.3.1 Substrate moisture before irrigation

6.3.1.1 Effect of substrate dryness on denitrification

When denitrification N loss was measured from pots of *E. pulcherrima* that contained dry peat substrate (20 vol.% H_2O), emissions were very low in comparison to those from moist peat (55 vol.% H_2O). In total, emissions from dry substrate hardly reached 1/3 of those from moist substrate (Fig. 1). Inspite of the rather long irrigation period of 2 hours, the dry substrate reached a much lower water content than the moist substrate. Obviously the water repellency caused by drying of the peat prevented water uptake, and thus reduced denitrification to a large extent. Consequently, it was observed that in the dry substrate denitrification took place already at a mean water

content of 59 vol.%, while N emissions in the moist substrate required a mean water content of 68 vol.% (Fig. 2).

Fig. 1 (N_2 +N_2O)-N emissions and water content of sieved peat substrate planted with *E. pulcherrima* with different substrate moistures before irrigation (5 vol.% C_2H_2)

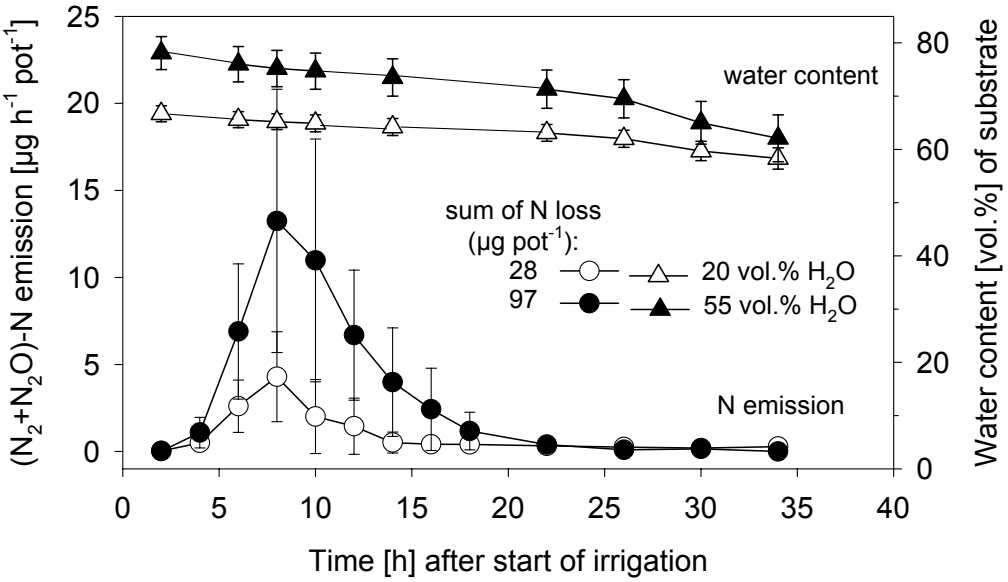
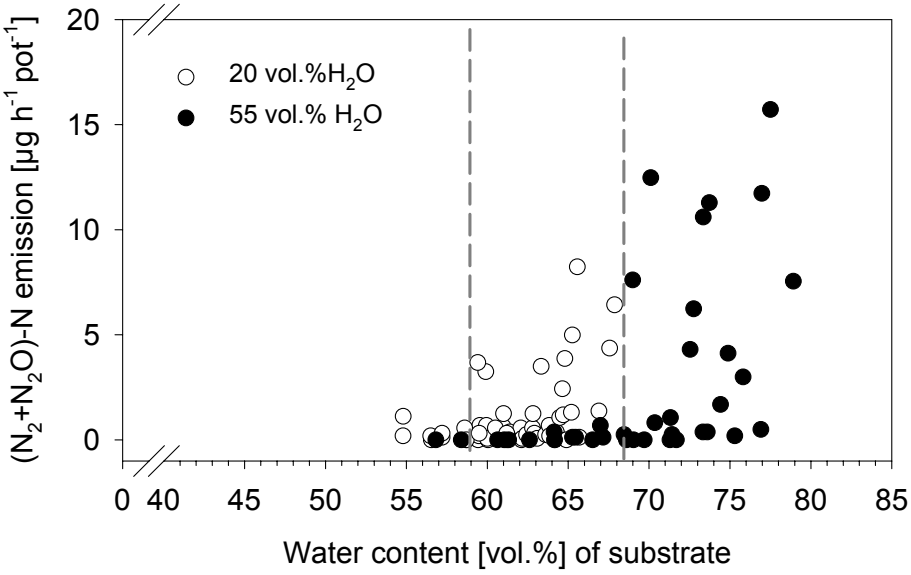
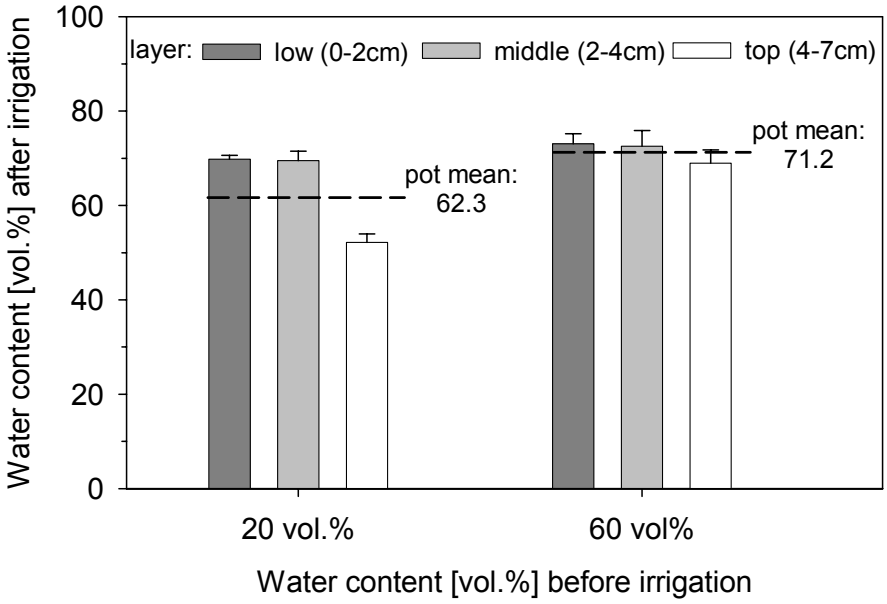


Fig. 2 (N_2 +N_2O)-N emissions depending on water content of sieved peat substrate planted with *E. pulcherrima* with different substrate moistures before irrigation (5 vol.% C_2H_2)



To investigate this difference in threshold values, water distribution within potted substrate was determined. When potted peat of low (20 vol.%) and high (60 vol.%) mean water content was divided into three horizontal layers after irrigation, it resulted that the water content of low and middle layer was only by about 3 vol.% lower in the dry substrate than in the moist substrate. Yet, the top layer of the dry substrate showed a significantly reduced water content which was 17 vol.% lower than that of the moist substrate (Fig. 3). Thus, the lower mean water content and threshold value of dry peat mainly resulted from decreased water content of the top substrate layer.

Fig. 3 Water content of substrate layers after flood irrigation as affected by mean water content of potted peat substrate before irrigation



6.3.2 Substrate compaction

6.3.2.1 Effect of substrate compaction on denitrification

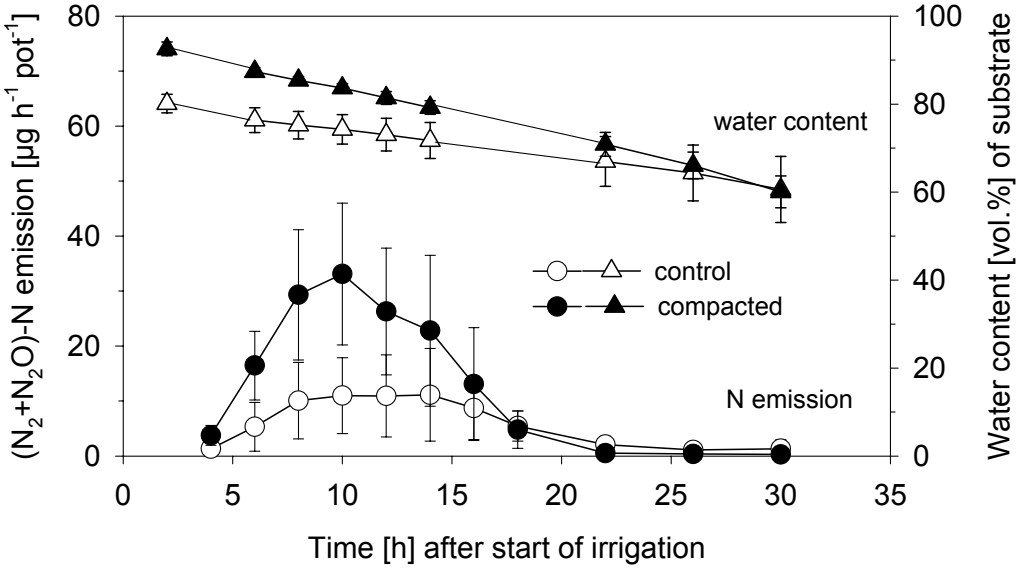
When denitrification was measured from compacted and uncompacted peat substrate planted with *P. zonale,* up to three times higher emissions resulted from the compacted treatment (Fig. 4). The compacted substrate reached a higher water content after irrigation than the control substrate. Although the water content of the

compacted substrate remained at a higher level throughout the experiment, the duration of denitrifying activity in both treatments was the same. In total, the compacted substrate produced two times higher N loss (Tab. 1). In contrast to higher (N_2+N_2O)-N emissions, the compacted substrate emitted less N_2O than the control substrate (Tab. 1). The N_2O:N_2 ratio of the control substrate was more than four times higher than that of the compacted substrate. Both, the high (N_2+N_2O)-N loss and the low N_2O emission indicate that anaerobiosis was stronger in the compacted substrate.

When N emissions were related to water content (Fig. 5), it appeared that in the compacted substrate denitrification took place when its mean water content exceeded 80 vol.%, while the control substrate already emitted N at about 66 vol.%.

When water distribution in compacted and uncompacted potted substrate was investigated, the compacted substrate showed higher water content in all substrate layers (Fig. 6). Thus, the higher mean water content of the compacted substrate presumably resulted from overall higher water capacity. To confirm this assumption, water and air capacity of compacted and uncompacted peat substrate were investigated.

Fig. 4 (N_2 +N_2O)-N emissions and water content of pots of *P. zonale* as affected by compaction which increased bulk density by 1/3 following irrigation (5 vol.% C_2H_2)



Tab. 1 Sum of (N_2 +N_2O)-N and N_2O-N emissions from pots of *P. zonale* with compacted and uncompacted substrate following irrigation (0 or 5 vol.% C_2H_2)

treatment	(N_2 +N_2O)-N emission [mg pot^{-1}]	N_2O-N emission [mg pot^{-1}]	N_2O:N_2 ratio
control	0.148 ± 0.084	0.060 ± 0.021	0.68
compacted	0.307 ± 0.122	0.040 ± 0.016	0.15

Fig. 5 (N_2 +N_2O)-N emissions depending on water content of pots of *P. zonale* as affected by compaction which increased bulk density by 1/3 (5 vol.% C_2H_2)

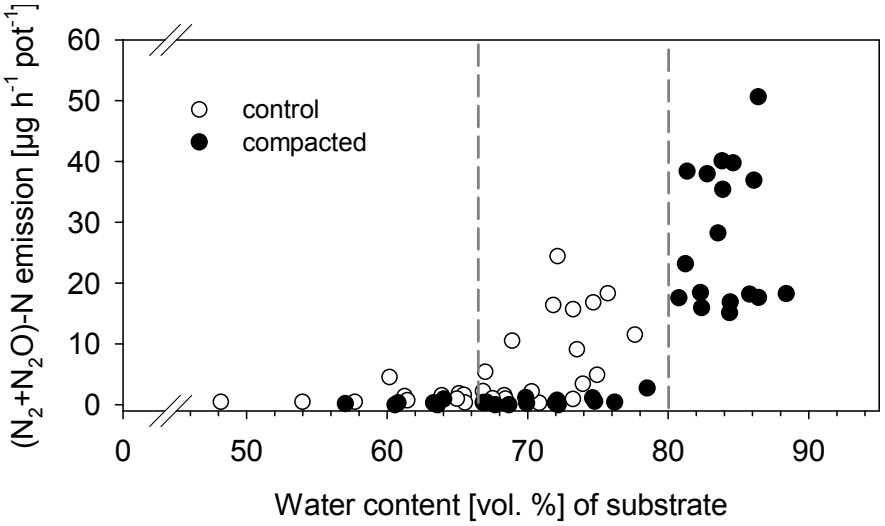
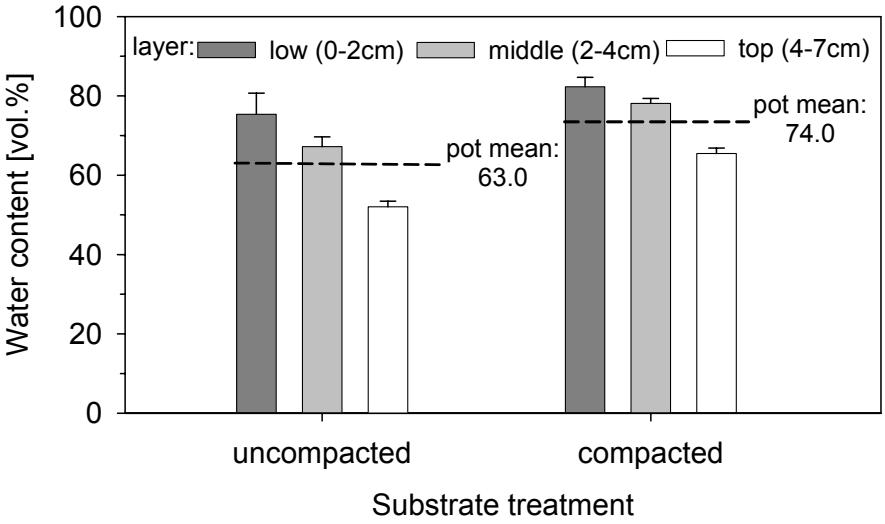


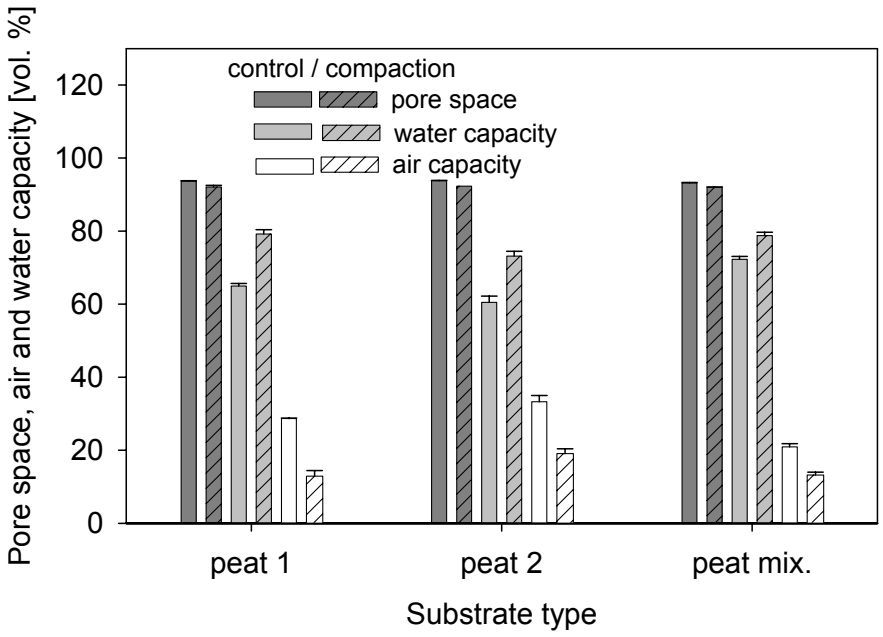
Fig. 6 Water content of substrate layers after flood irrigation as affected by compaction which increased bulk density of potted substrate by 1/3



6.3.2.2 Effect of compaction on substrate characteristics

Three peat substrates were used to study the effect of compaction on pore volume, water and air capacity. The substrate used for the previously described denitrification measurement (Fig. 4, 5, Tab. 1) was called ‘peat mix’. In all substrates the total pore space was hardly affected by compaction, while water and air capacity changed significantly (Fig. 7). Water capacity was increased by compaction, and air capacity was reduced. These results confirm the differences in water content between treatments that were observed during denitrification measurement (Fig. 4).

Fig. 7 Pore volume, water capacity, and air capacity as affected by compaction which increased bulk density by one third



6.3.3 Sieving of substrate

6.3.3.1 Effect of substrate sieving (5 mm) on denitrification

Plants of *P. zonale* were cultivated in white peat substrate, which was sieved or not sieved prior to planting. When denitrification was measured from these pots, the sieved substrate produced higher N emissions than the unsieved substrate (Fig. 8). While the duration of denitrifying activity was similar in both treatments, N emissions

from the sieved substrate reached a higher maximum and started to decrease later than emissions from the unsieved substrate. Although the course of N emissions showed obvious differences, water contents of the substrates were about the same. The course of N emissions indicated that oxygen deficiency lasted longer in the sieved substrate because the decrease of emissions took place later than in the control substrate.

When (N_2 +N_2O)-N emissions were related to water content of the substrate (Fig. 9), it appeared that emission of gaseous N from the unsieved substrate required a mean water content of about 71 vol. %, while the sieved substrate already denitrified at a mean substrate water content of 68 vol. %. Measurement of water distribution after irrigation in sieved and unsieved potted substrate showed similar water contents in each of the substrate layers independent of the treatment (Fig. 10). Thus, water distribution did not help to explain the observed differences in denitrification N loss.

For further investigation, the effect of sieving on air and water capacity of peat substrate was analyzed.

Fig. 8 (N_2 +N_2O)-N emissions from pots of *P. zonale* with sieved (5 mm) and unsieved substrate (brand 2) following irrigation (5 vol.% C_2H_2)

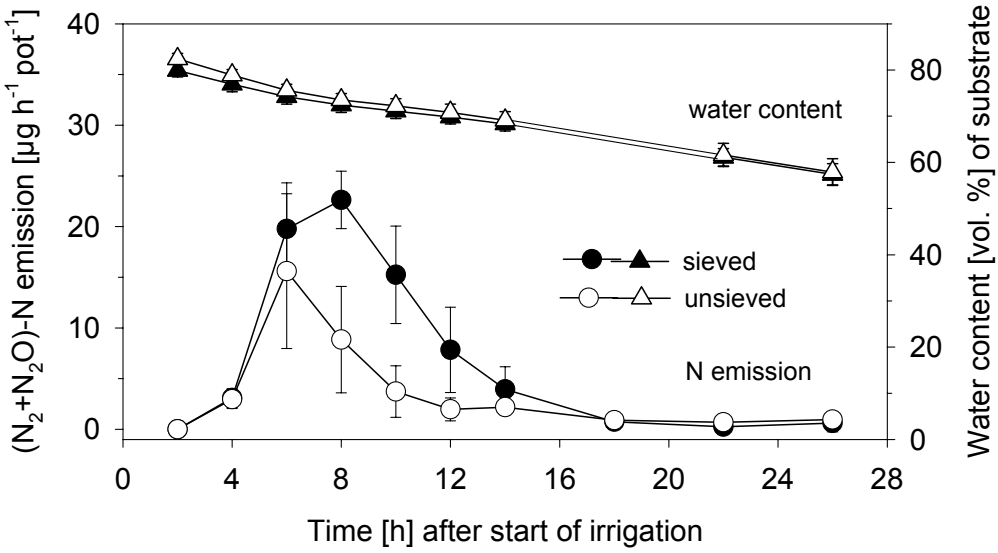


Fig. 9 (N_2 +N_2O)-N emissions depending on water content of sieved (5 mm) and unsieved substrate (brand 2) planted with *P. zonale* (5 vol.% C_2H_2)

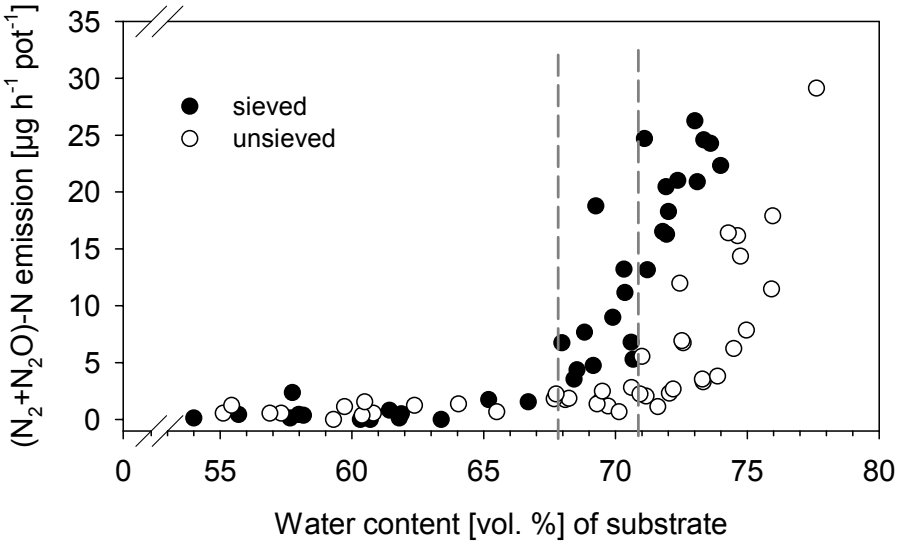
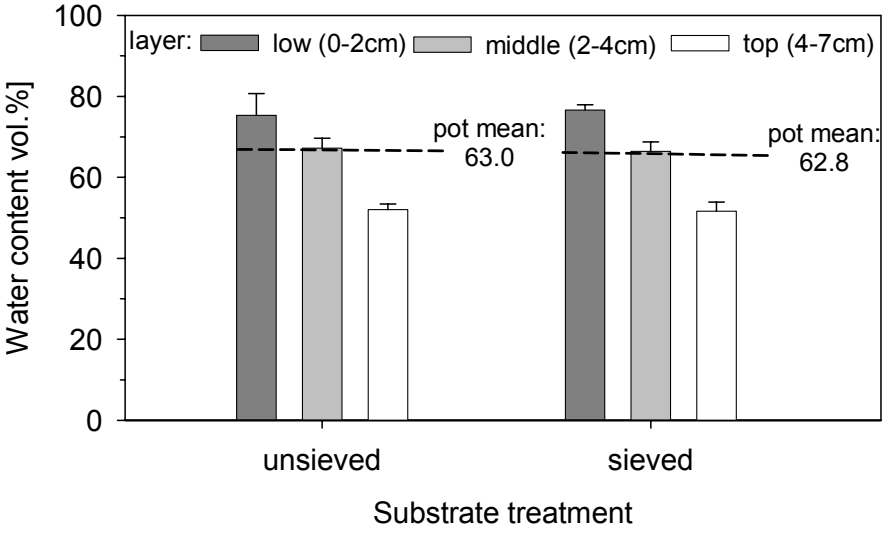


Fig. 10 Water content of substrate layers after flood irrigation as affected by sieving of potted substrate

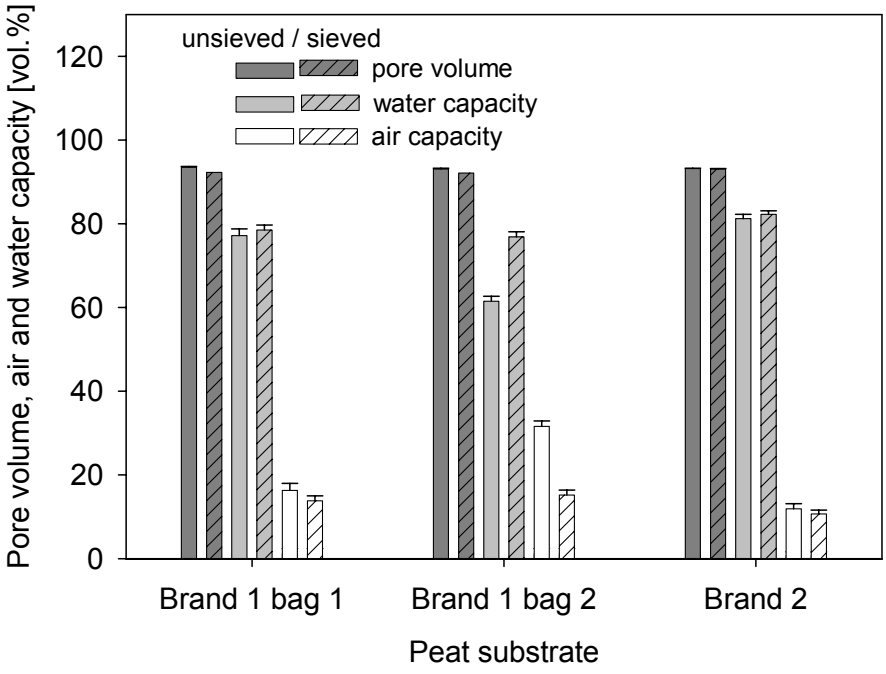


6.3.3.2 Effect of sieving (5mm) on substrate characteristics

During measurement of physical substrate characteristics, it was observed that even peat substrates of the same brand were differently affected by sieving. To investigate

these differences, three sieved (5 mm) and unsieved white peat substrates of two brands were analyzed by ‘Quick’-method (Fig. 11). The peat substrate used for denitrification measurement (Fig. 8, 9) was of brand 2. The total pore volume was uniform between all peats and in all cases it was only marginally reduced by sieving. Water and air capacity remained nearly unchanged when peat of brand 1 bag 1 and brand 2 was sieved. Only small decreases in air capacity and increases in water capacity could be observed. Unsieved substrate from brand 1 bag 2, in contrast, showed an about 15 vol.% lower water capacity and a correspondingly higher air capacity than the other two substrates. After sieving this difference vanished. Presumably, peat from bag 2 was of a coarser type than the other peats and thus, it was more affected by sieving.

Fig. 11 Pore volume, water capacity, and air capacity of unsieved and sieved (5 mm) peat substrate from three different bags



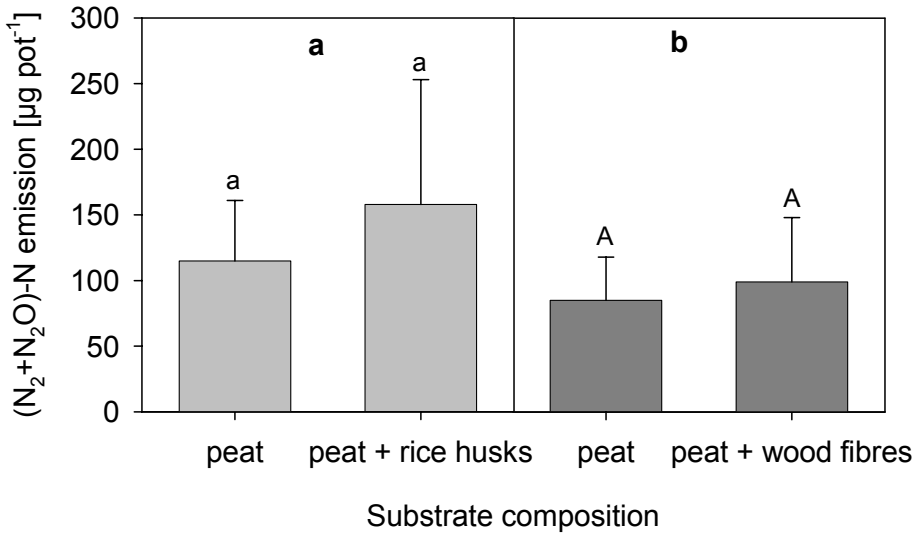
Although sieving hardly changed water and air capacity of the peat used for denitrification measurement (brand 2), denitrification N loss was strongly increased by sieving (Fig. 8). So, either the observed small changes in air and water capacity were enough to promote denitrification, or a change in substrate characteristics took place that could not be measured by the applied methodology.

6.3.4 Composition of substrate

6.3.4.1 Effect of substrate composition on denitrification

To investigate the effect of substrate composition on denitrification, measurements were conducted with pots of *P. zonale* grown in sieved peat mixed with rice husks (Fig. 12 a) and in sieved peat mixed with wood fibres (Fig. 12 b). In both cases, control treatments consisted of pure sieved peat. Addition of rice husks or wood fibres lead to about the same N loss than from pure peat substrate. The slight increases in N loss that were observed with the peat mixtures were statistically non-significant and presumably a product of variability. These results were considered surprising as both materials, wood fibres and rice husks, were expected to improve substrate aeration and thus, decrease denitrification N loss. In case of rice husks, it was observed after denitrification measurement that the outer appearance of the husks had changed. Possibly, rice husks broke down during the cultivation period which lastet about 12 weeks in this experiment.
To clarify the effect of wood fibres and rice husks on air capacity of peat substrate, substrate mixtures were analyzed in a laboratory experiment.

Fig. 12 a+b (N_2+N_2O)-N emissions from pots of *P. zonale* planted in sieved peat and in 70 vol.% sieved peat and 30 vol.% rice hulls or wood fibres (5 vol.% C_2H_2)

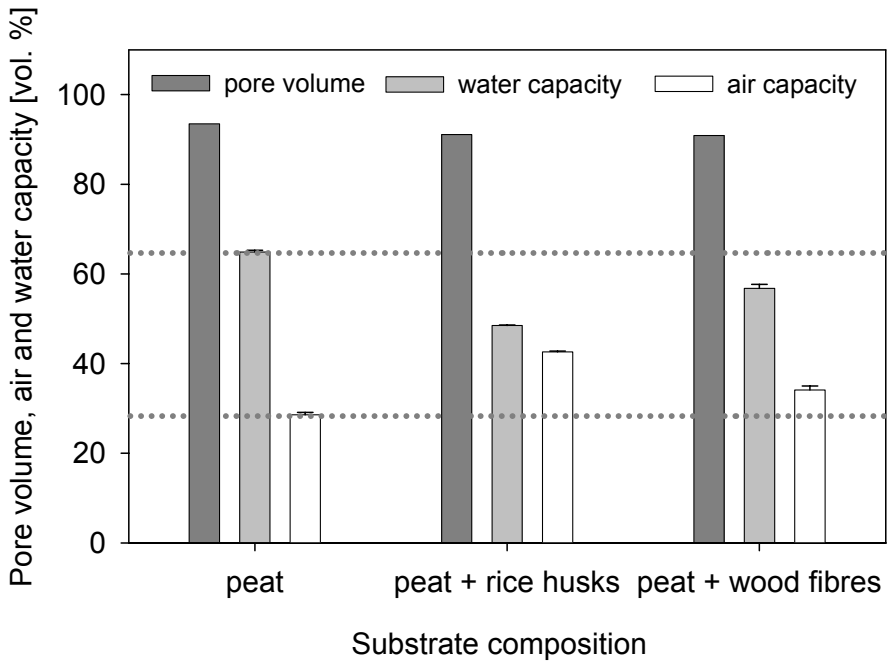


Similar letters indicate non-significant differences between treatments (Tukey; α=0.05; n=6)

6.3.4.2 Effect of additives on substrate characteristics of peat

Inspite of similar denitrification N loss, air capacity of peat substrate was increased by both, wood fibres and rice husks (Fig. 13). The addition of wood fibres to sieved peat increased air capacity by about 5 vol.% relative to peat, while addition of rice husks increased air capacity even by one third (14 vol.%) compared to pure peat.
These results do not correspond to the observed denitrification N loss, which was similar between peat and peat mixtures (Fig. 12 a+b). Like mentioned before rice husks, and maybe also wood fibres, were possibly decomposed during the cultivation period and thus, might have lost their positive effect on substrate air capacity.

Fig. 13 Pore volume, water capacity, and air capacity of peat and peat mixtures (100 vol.% sieved peat; 70 vol.% sieved peat + 30 vol.% rice hulls or wood fibres)



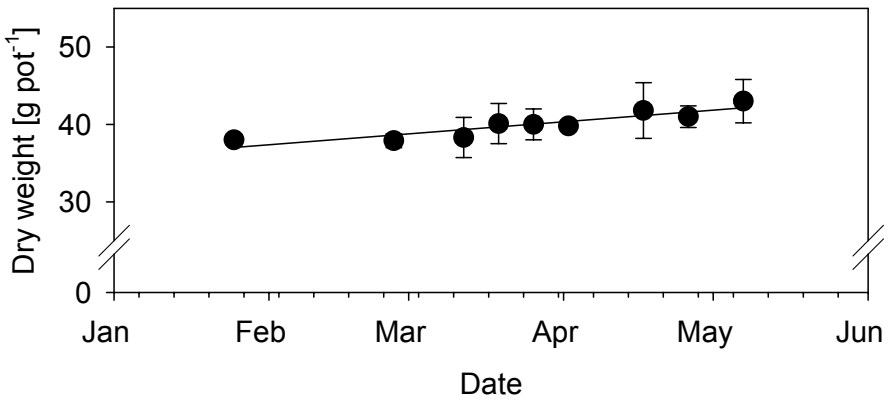
6.3.5 Planting of substrate

Laboratory measurements of pore volume, air and water capacity were done with unplanted substrate, while denitrification was measured from planted substrate. As treatment effects on denitrification N loss did not always correspond to those on substrate air capacity, it was questioned if planting altered substrate characteristics

and thus, limited transferability of results from laboratory to greenhouse, and vice versa.

This speculation was encouraged by the observation that the dry matter per pot, which was determined after each denitrification measurement, slightly increased with time during cultivation, although pots were filled and planted uniformly on the same day (Fig. 14). It was suspected that this was an effect of root growth. To clarify if root growth affected substrate properties, pore volume, air and water capacity of planted and unplanted substrate were determined.

Fig. 14 Increase of dry weight per pot during cultivation of *P. zonale* in a sieved peat substrate

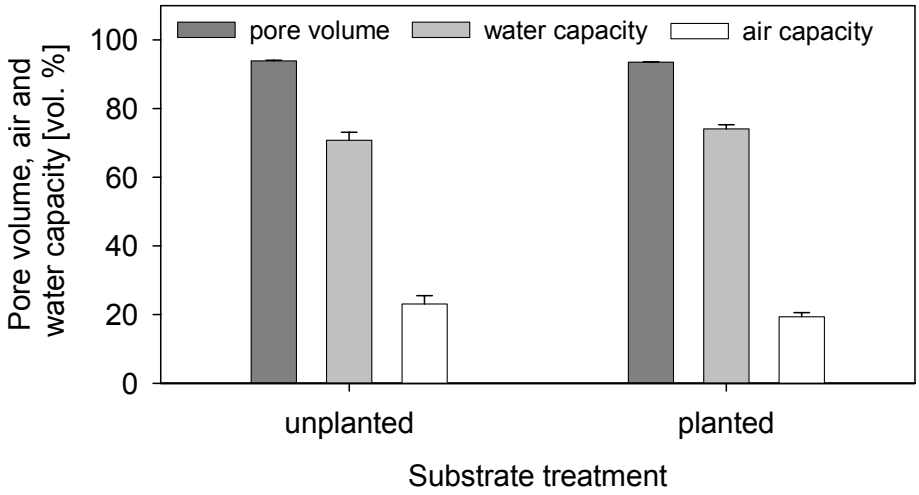


6.3.4.1 Effect of plant growth on substrate characteristics

Test cylinders with planted and unplanted peat substrate were cultivated in a greenhouse for eight weeks. After this period water and air capacity showed only little differences between treatments. The planted substrate showed a 4 vol.% lower air capacity relative to the unplanted substrate, while total pore space was the same in both treatments (Fig. 15). Plant roots increased the dry weight of the substrate by about 5 g and reduced shrinking of the substrate by 9 mL compared to the unplanted substrate (data not shown).

All in all, changes in physical substrate properties by planting were considered small enough to transfer laboratory results obtained with unplanted substrate to planted substrate of greenhouse experiments.

Fig. 15 Pore volume, air capacity, and water capacity of unplanted and planted unsieved peat substrate after eight weeks of cultivation



6.4 Discussion

6.4.1 Effect of substrate properties on denitrification

6.4.1.1 Substrate moisture

Denitrification N loss from dry peat was not even 1/3 of that from moist peat (Fig. 1). N emission rates were much lower and the denitrifying activity ended sooner in the dry than in the moist peat. This was attributed to the lower water content that was reached by the dry substrate after irrigation relative to the moist substrate (Fig. 1, 3). As a decrease in water filled pores corresponds to an increase in air filled pores, it is very likely that denitrification in drier substrate was repressed by oxygen availability.
The effect of substrate moisture on water content after irigation was also discussed in Chapter 4. There, experimental results were presented which prove a close relationship between water content of peat substrates before and after irrigation. The problem of water repellency of dried peat was often described in literature (Niggemann 1970, Valat et al. 1991, Michel et al. 1999). It is reported to be related to chemical (humic polymers) as well as physical (shrinkage, adhesion) characteristics of peat. Hydrophobia after drying was also described for soils, where it is generally related to soil organic matter (Ellies et al. 2003, Wallis and Horne 1992, Ma'Shum et

al. 1988). Still, like discussed in Chapter 4, its effect on denitrification has not received much attention in literature.
As differences in water content were shown to provoke differences in denitrification N loss, it was considered most important to conduct denitrification measurements only with substrates of homogenous water content.

6.4.1.2 Substrate compaction

Denitrification N loss from compacted substrate was significantly higher than from uncompacted substrate (Fig. 4, Tab. 1). In contrast to this, N_2O-N emissions were lower from compacted than from uncompacted substrate. Already during denitrification measurement the compacted substrate showed higher water content than uncompacted substrate. The difference in water content was present throughout the substrate, irrespective of the height of substrate layers in pots (Fig. 6). This was confirmed by laboratory measurement, where compacted substrate proved to have decreased air capacity and increased water capacity relative to the control (Fig. 7).
Reduction of air filled pores is a common consequence of soil or substrate compaction. Richard et al. (2001) analyzed pore size distribution of agricultural soils and showed that the total porosity was relatively little affected by soil compaction, because the amount of macropores destroyed was mostly compensated for by an increase of micropores. The same was stated by Kooistra and Tovey (1994). In their review on soil compaction and soil aeration, Stepniewski et al. (1994) presented many examples for reduced soil aeration due to compaction.
Lack of oxygen is one of the crucial prerequisites for denitrification, consequently the promoting effect of soil compaction on denitrification has repeatedly been reported with regard to agricultural soils. Torbert and Wood (1992) investigated the effect of soil compaction on N loss and observed a more than 3-fold increase of N loss when the soil bulk density was increased from 1.4 to 1.8 Mg m^{-3} soil. They suggested that soil compaction reduced O_2 diffusion by restricting continuity of air-filled pores and decreasing the amount of larger pores. Soil compaction by tractor traffic was found by Bakken et al. (1987) to cause 2 to 4-fold higher N emissions relative to uncompacted soil. Apart from observing increasing N loss at increasing soil compaction, Walenzik and Heinemeyer (1990) found that the emission of N_2O also increased with compaction but that the ratio N_2O:N_2 decreased. The increased reduction of N_2O in compacted soils may be related to the lower diffusion coefficient

of gases at high bulk density. When the escape of N_2O from the site of reduction to the atmosphere is delayed, the chance for reduction to N_2 is increased (Smith 1980). In general, it is assumed that the share of N_2O decreases when conditions for denitrification, like oxygen deficiency, become more favorable (Tiedje 1988).

6.4.1.3 Sieving of substrate

Denitrification N loss from sieved (5 mm) peat substrate was higher than from unsieved peat substrate (Fig. 8). The water content of the substrates was nearly the same after irrigation, and also throughout the experiment. Yet, emissions from the sieved substrate lasted longer and reached a higher peak. This indicated increased oxygen deficiency in the sieved relative to the unsieved peat substrate. But, measurement of water distribution within the potted substrate showed no difference between sieved and unsieved substrate (Fig. 10). Also, water and air capacity of the peat substrate used for denitrification measurement (brand 2) showed no change after sieving (Fig. 11).

In contrast to this, air capacity was described in literature to decrease when the fraction of small substrate particles increased (Scharpf 1997, Limbers and Rehme 1997, van Schie 1999, Verhagen 1997). These controversial observations raised the question wether laboratory measurements with unplanted substrates were transferable to planted substrates cultivated under greenhouse conditions. Yet, when pore volume, air and water capacity of planted and unplanted substrate was compared, no significant differences could be found (Fig. 15). Thus, it was concluded that laboratory measurements of substrate characteristics were reliable.

Still, other observations reported in literature might explain the high denitrification N loss of the sieved relative to the unsieved substrate. Prasad and O'Shea (1999) found out that the particle sizes of peat were related to the degree of its breakdown with fine peat breaking down more than coarse peat. During an incubation period of 15 month they observed decreases of airspace and volume reductions especially in substrates of finer particle size. Michiels et al. (1993) observed higher shrinkage of fine substrate than of coarse substrate during nine month exposition to ebb/flood irrigation. So, possibly, sieved and unsieved substrate changed differently during the cultivation period, although the initial values for pore volume and air capacity were the same. This could explain the difference in denitrification N loss. Additionally, Torbert and Wood (1992) concluded that within the same level of water-filled pore

space changes in pore structure could contribute to an increase of anaerobic microsites. This, they referred to compacted soils, but it may also apply to sieved substrate.

Curiously, the effect of substrate sieving was not uniform among substrates. In addition to the substrate used for denitrification measurement (brand 2), two more white peats were analyzed with and without sieving with regard to water and air capacity (Fig. 11). Only one of three tested substrates (brand 1 bag 2) showed changes after sieving. Its air capacity, which was significantly higher than that of the other two samples before sieving, was reduced by about half and thus, was about on one level with the other samples. Probably, the two substrates that showed little change in air capacity after sieving were of a finer type and were consequently not altered in the amount of water holding pores.

6.4.1.4 Composition of substrate

Different media are added to horticultural substrates, either to replace peat or to improve substrate characteristics. The effect of two of these, wood fibres and rice husks, on air capacity and denitrification of a peat substrate was investigated. The components, wood fibres or rice husks, were separately added to sieved white peat to achieve mixtures of 70 vol.% : 30 vol.%. Denitrification N loss of the mixes was about the same than that of plain peat (Fig. 12 a+b). This was surprising as both materials were expected to increase air capacity. This assumption was confirmed by measurement of air and water capacity, which showed that the substrate mixes had a significantly higher air capacity than the plain peat substrate, while the total pore volume was the same for all substrates (Fig. 13). As the outer appearance of rice husks had changed during cultivation, it was suggested that decomposition reduced the positive effect on air capacity and thus, denitrification was not reduced relative to plain peat substrate. For wood fibres the same might apply, as their breakdown during cultivation was repeatedly reported in literature. This lead to loss of volume, decrease of air capacity, and increase of water capacity (Prasad and O'Shea 1999, Leinfelder et al. 1995, Fischer et al. 1993, Rest et al. 2002). As water contents of the substrate mixtures were the same during denitrification measurement as of the pure peat (data not shown), it seemed that in the presented study the negative development of air capacity was either not present or it was equal to that of the sieved peat substrate. Like discussed in Chapter 6.4.2.3, the air capacity of sieved

peat presumably decreased during cultivation of plants. Apparently, wood fibres and rice husks were not able to compensate this negative development.
Thus, the effect of substrate components on denitrification seemed to depend on their stability during the cultivation period. Breakdown or decomposition of growing media is likely to favor denitrification by decreased air capacity and increased carbon availability which accelerates oxygen consumption.

6.4.2 Threshold values of mean substrate water content for denitrification

In this chapter threshold values of mean water content have been presented below which denitrification was inhibited (Fig. 2, Fig. 5, Fig. 9). As water content is the counterpart of air content, it was assumed that return of air into the substrate and availability of oxygen put an end to denitrifying activity when water content decreased below a specific value. Such threshold values have also been reported for denitrification in soils (Ryden 1983, Shelton et al. 2000, Aulakh et al. 1991b, de Klein and van Logtestijn 1996, Weier et al. 1993).
As oxygen deficiency in soil or substrate is unwanted, thresholds for denitrification could serve as indicators for substrate aeration. Unfortunately, threshold values of the presented study were not uniform, but influenced by physical substrate characteristics (Tab. 2).

Tab. 2 Threshold values of substrate mean water content for denitrification as presented in this study

Substrate	Source	Threshold value [vol.%] of mean water content
Sieved white peat (5 mm mesh), moist	Fig. 2	68
Sieved white peat (5 mm mesh), dry	Fig. 2	59
Unsieved, uncompacted white peat	Fig. 5	66
Unsieved, compacted white peat	Fig. 5	80
Unsieved white peat	Fig. 9	71
Sieved white peat (5 mm mesh)	Fig. 9	68

The substrate that was relatively dry before irrigation showed a lower threshold value than the substrate that was moist before irrigation (Fig. 2). The threshold value of mean water content for compacted peat was distinctly higher than that of non-compacted peat (Fig. 5), but sieved substrate in contrast, showed a slightly lower threshold value than unsieved peat (Fig. 9). In case of compacted and dry substrate, water distribution within potted substrate showed that differences in mean water content after irrigation relative to the control treatment were mainly due to changes in water content of the top substrate layer (Fig. 3, 6). The top layer of the dry substrate had a 17 vol.% lower water content after irrigation than that of the moist substrate (Fig. 3). Substrate compaction increased the water content of the top layer after irrigation by 14 vol.% relative to the control treatment. Because of its high volume (about 145 mL per 340 mL pot), the water content of the top layer was well reflected in the mean water content per pot. Yet, in another study it was shown that denitrification mostly took place in the lower substrate layers (Chapter 7). Although the top layer of potted substrate did not contribute to denitrification N loss, it did influence mean water content and thus, cause shifts in threshold values for denitrification.

In case of sieved and unsieved substrate, analysis of unplanted substrate did not reveal differences in water capacity (Fig. 11) or water distribution between layers of potted substrate (Fig. 10). It may be speculated that the difference of 3 vol.% in threshold values (Fig. 9) was caused by uneven mean water content of sieved and unsieved substrate before irrigation. Although the difference was very small, it proceeded throughout most of the denitrification measurement with sieved substrate showing up to 2.5 vol.% lower water content than unsieved substrate (Fig. 8). As threshold values were derived from mean water content, this difference was likely to be reflected in threshold values. Its effect on water capacity or water distribution could not be observed by analysis of unplanted substrate as for these measurements only substrate with homogenous water content was used.

Thus, it was concluded that because of its high variability the mean water content was not apt for deduction of threshold values for denitrification.

In literature, varying threshold values between soils were ascribed to soil texture, with finer texture causing lower threshold values (de Klein and van Logtestijn 1996). Aulakh et al. (1991b) showed that threshold values for differently textured soils were uniform when expressed as water filled pore space (wfps) instead of water content.

But here, the total pore volume of peat substrates usually remained unchanged, so differences were also present if values were expressed as wfps. Prade and Trolldenier (1988) found a higher threshold value of air content at increased soil organic matter content, which they related to increased oxygen consumption by microbia. So, also in soils there seem to be various factors that influence threshold values. Some authors consider measurement of gas diffusivity a better tool than air or water filled pore space for assessing the aeration status of a soil (Zausig et al. 1993, Allaire-Leung et al. 1999).

6.4.3 Summary

The wettability of peat, which proved to depend on substrate water content, showed a strong effect on denitrification N loss. Dry substrate produced less N emissions after irrigation than comparatively moist substrate. Although the difference in water content between the substrates chosen for study was big, it is considered probable that also lesser differences in water content between substrates influence denitrification N loss and thus, complicate denitrification measurement.

Sieving as well as compacting peat substrate increased denitrification N loss. This was mainly attributed to decreased air capacity. While the compacted peat showed increased water and decreased air capacity already before planting (‘Quick’-method), the sieved substrate presumably deteriorated during cultivation.

Mixing sieved peat with rice husks or wood fibres (30 vol.%) increased air capacity as measured by ‘Quick’-method. Still, observed N emissions from the mixed substrate were at least as high as from plain peat. It was suggested that rice husks as well as wood fibres broke down and decomposed during the cultivation period and thus, lost their positive effect on substrate aeration.

It was observed that threshold values of mean water content for denitrification changed with substrate characteristics. Thus, threshold values of mean water content were not considered usable as indicators of oxygen deficiency or denitrifying activity.

7. Localization of denitrifying sites

7.1 Introduction

The water content of the substrate and its dynamics proved to be most important for denitrification in potted and planted peat substrate (Chapter 4). Yet, water is not distributed uniformly within potted substrate. Modelling of water content showed that highest values are to be expected close to the pot bottom and that water content decreases with increasing distance of substrate layers from the bottom (Fonteno 1989). In experiments with 18 cm high soil columns McCarty et al. (1999) found decreasing oxygen concentrations with increasing soil depth, especially when air porosity was reduced. They, as well as Shelton et al. (2000), observed higher N_2O production near the bottom of soil cores than near the surface.

Consequently, it was questioned if a gradient in water content lead to zones of differing denitrifying activity within potted substrate. For more precise evaluation of the denitrifying potential of substrate layers, measurement of redox potential was considered useful. The redox potential is a measure of the electron availability. It results from biochemical reduction processes and is closely related to the availability of oxygen. Under aerobic conditions, microbia generally use oxygen as electron acceptor, while during anaerobiosis oxidized inorganic soil components are being reduced. When oxygen is depleted, nitrate is used next as electron acceptor, followed by ferric iron and sulfate (Patrick and Mahapatra 1968). As the reduction of the oxides is more or less sequential, the level of redox potential can be used as an indicator for the electron acceptor that is currently being used (Ottow and Fabig 1984). Thus, knowledge of redox potential is useful for localization of denitrifying sites, because nitrate reduction is accompanied by a decrease of redox potential (Flessa and Beese 1995, Ryden and Lund 1980).

To localize the origin of gaseous N emissions within pots, experiments were conducted to investigate the dynamics of water distribution in potted and planted horticultural substrate. Further, a denitrification experiment was combined with measurements of redox potential to characterize zones of high denitrifying activity.

7.2 Materials and Methods

7.2.1 Denitrification measurement

7.2.1.1 Experimental setup

Flow-through chambers like described in Chapter 2.2.2 were used for measurement of denitrification from planted substrate.
Before the start of the experiment substrates were pretreated with acetylene (C_2H_2) within the chambers as described in Chapter 7.2.2.
After pretreatment with C_2H_2 pots were flood irrigated within the chambers.
The irrigation event was defined start of the experiment. Air samples were taken with syringes topped with gauge needles from silicon tubes at the air outlet of the chambers every one to four hours until N_2O emissions ceased.

7.2.1.2 Application of C_2H_2

For determination of (N_2+N_2O)-N emissions by denitrification, 5 vol.% C_2H_2 was added to the chamber atmosphere. To guarantee immediate inhibition of N_2O reduction to N_2 substrates were pretreated for two hours with 5 vol.% C_2H_2 prior to irrigation.

7.2.1.3. Duration of fertigation and composition of fertigation solution

For denitrification measurement, pots were fertigated with 1 L of a solution containing 150 mg NO_3-N L^{-1}. The irrigation solution was released by an opening at the bottom of the chambers after two hours.
During the cultivation period plants were fertigated with 1g L^{-1} of a full compound fertilizer (Flory3, Euflor) according to horticultural practice.

7.2.1.4 Substrates

A commercial, full fertilized white peat substrate was used to investigate the distribution of water within potted substrate. Denitrification and redox potentials were measured from pots of a commercial, full fertilized peat substrate composed of white peat and coir (peat mix).

7.2.1.5 Plant material

Experiments were conducted with plants of *Pelargonium zonale* 'Grand Prix'. Plants were propagated by cuttings and rooted in small peat nuggets (Jiffy7) for two weeks. Then plants were potted into plastic pots (340 mL) and cultivated for at least four weeks to guarantee rooting of the substrate. For each experiment plants of the same species and the same set were used, i.e. plants were of the same age and of similar growth.

7.2.2 Measurement of redox potential

7.2.2.1 Fabrication and testing of electrodes

A 1 cm piece of platin/iridium wire (90:10; 0.4 mm diameter) was soldered to plastic coated copper wire. The joint was sealed with a two component adhesive and coated with shrinkable tubing. Both ends of the tubing were sealed with a two component glue. Prior to use all electrodes were tested in A. dest. and those showing variability in readings were discarded.

7.2.2.2 Application of electrodes

One day before the experiment, pots of *P. zonale* were each equipped with nine electrodes. The electrodes were carefully inserted into the substrate and it was aimed to position three of them at 1 cm, 3 cm, and 5 cm height from the pot bottom. The final position of the electrodes was verified and noted after the experiment.

7.2.3. Analytical procedures

7.2.3.1 N_2O

The analysis of N_2O in all air samples was performed by a gas chromatograph (Chrompack 9001) with an electron capture detector (ECD) according to a method described by Mosier and Mack (1980).

7.2.3.2 Water content

During denitrification measurement every one to four hours pots were taken from the chambers and weighed. At the end of the experiment plants were harvested, the fresh weight of the shoot and of the rooted substrate was determined. Then, the substrate was dried at 105°C and weighed again. Water content (vol.%) was calculated from pot volume, fresh weight of shoot, dry weight of rooted substrate, and fresh weight during the experiment.
The water content of unplanted potted substrate was determined by weighing the substrate before and after drying at 105°C .

7.2.3.3 Measurement of redox potential

For measurement of redox potential from pots of *P. zonale*, a reference electrode (calomel) was inserted into the substrate surface. The potential difference between the reference electrode and the platin electrodes was read off a potentiometer (pH-meter). To these values the potential of the reference electrode (247 mV) was added to calculate the redox potential (Eh) (Böttcher and Strebel 1985).

7.2.3.4 Statistics

Statistics were performed by use of the SAS package. All measurements were conducted with five replications per treatment.

7.3 Results

7.3.1 Distribution of water in potted and planted peat substrate

To comprehend vertical distribution of water within potted substrates, peat substrate planted with *P. zonale* was harvested at different time intervals following flood irrigation and it was divided into three layers (Fig. 1). As pots were irrigated by flooding, the lowest substrate layer maintained the highest water content throughout the whole experiment. The lowest water content was always found in the top layer, while the water content of the middle layer stayed in between. Right after irrigation

the differences between layers were greatest and reached up to 16 vol.%, but then they decreased steadily until after 10 hours they amounted to less than 5 vol.%. Thus, the gradient of water content between substrate layers was dynamic and presumably resulted from plant water uptake.

Fig. 1 Water content of planted peat substrate after irrigation depending on substrate layer in a 340 mL pot

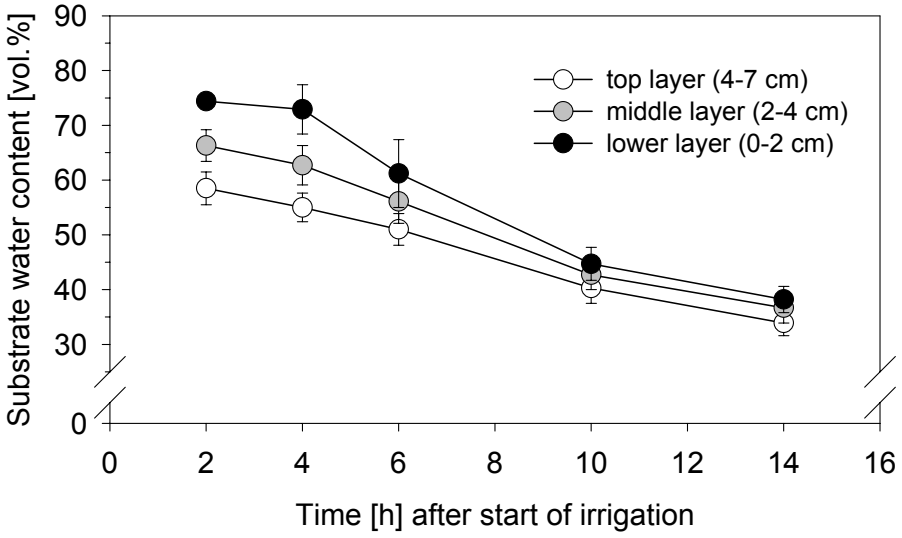
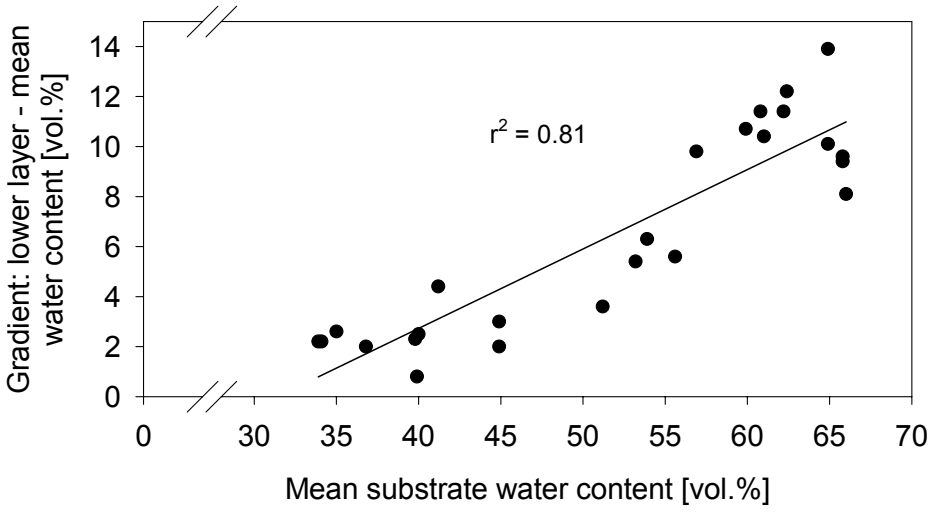


Fig. 2 Gradient between water content [vol.%] of lower substrate layer (0-2cm from pot bottom) and mean substrate water content [vol.%] of peat planted with *P. zonale*



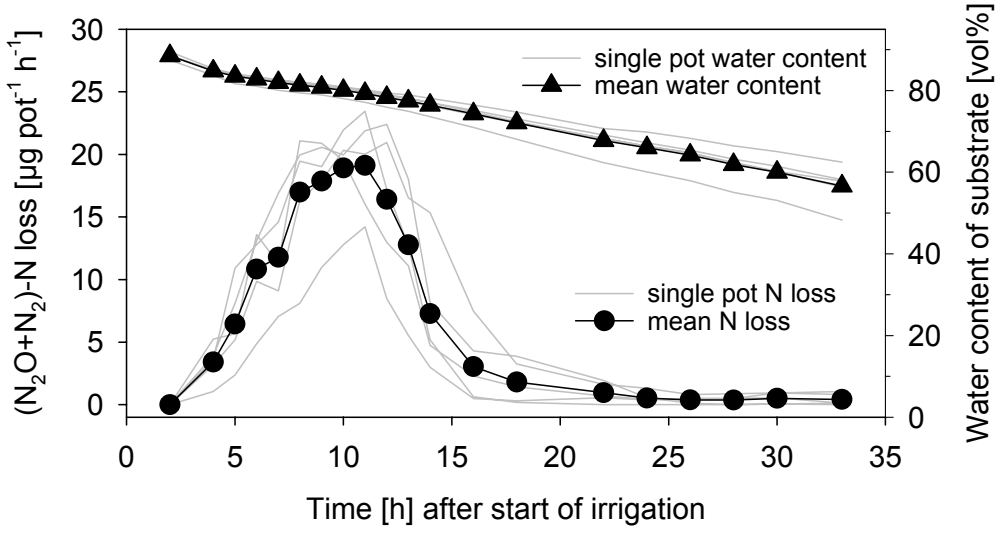
Because of its comparatively high water content, the lower substrate layer was thought to offer best conditions for denitrification. Its water content was permanently above the mean water content of the substrate (Fig. 2), especially at high mean water contents.

With regard to denitrification measurement the presented results imply, that best conditions for denitrification were offered right after irrigation and by the lower substrate layer. There, strongest anaerobiosis was to be expected within the pot because of highest water content. To confirm these findings and to further localize sites of denitrifying activity, N emissions and redox potential were measured simultaneously from pots of peat mix substrate planted with *P. zonale*.

7.3.2 Denitrification N emissions and redox potentials in planted peat substrate after irrigation

When redox potential and denitrification were measured simultaneously from substrate planted with *P. zonale*, denitrification N loss showed the typical high variability between replications (Fig. 3). Still, the mean substrate water content, which proved to have a strong influence on denitrification in horticultural substrates (Chapter 4), was quite uniform.

Fig. 3 Water content and (N_2+N_2O)-N loss from five pots of *P. zonale* planted in a peat mix substrate

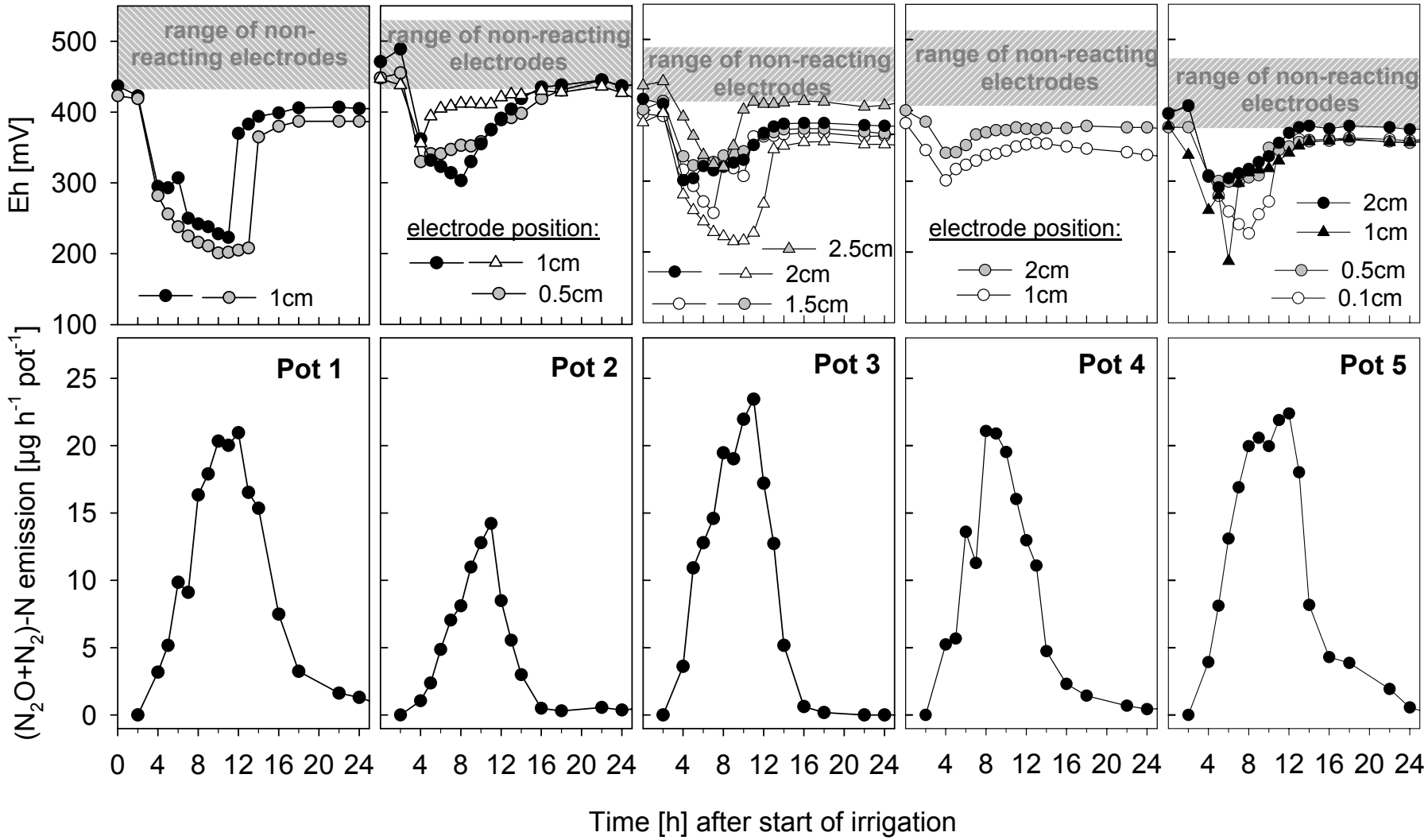


The redox potentials in the substrate proved to be as individual as denitrification N loss. Compression of data was hardly possible without losing quality and so, it was decided to present redox potentials and denitrification N loss per pot (Fig. 4). In each pot, there were electrodes that maintained a stable reading throughout the experiment. In their vicinity there was obviously no change in redox potential and they were summarized as ‘non-reacting electrodes’. Mostly, these electrodes were located in upper substrate layers, but there were also some close to the pot bottom.

The electrodes that did indicate changes in redox potential were all located close to the pot bottom and no farther than 2.5 cm from it. They mostly showed high synchrony with N emissions, especially at the onset of denitrification after 4 hours. The maximum of N emissions often coincided temporally with the minimum of redox potentials. Only at the end of denitrifying activity, the increase of redox potentials was quicker than the decrease of N emissions. This was probably due to incomplete control of all denitrifying sites by the few electrodes that were applied. The amount of reacting electrodes per pot showed high variability and did not necessarily correspond to the height of N emissions (e.g. Fig. 4, Pot 4). This indicates that positioning of the electrodes in a surrounding with changing redox conditions during measurement was a matter of random.

All in all, the measurement of redox potential showed that within a substrate volume of only 340 mL, there was high variability of redox conditions. It also confirmed that in an ebb/flood irrigation system denitrification N loss was most likely to originate from the lower substrate layers.

Fig. 4 Redox potentials and (N_2O+N_2)-N emissions of a peat mix substrate planted with *P. zonale* following irrigation (5 vol.% C_2H_2) (electrode position measured from the pot bottom)



7.4 Discussion

7.4.1 Distribution of water in potted and planted peat substrate

Previous studies (Chapter 4 and 6) as well as many reports in literature (e.g. Shelton et al. 2000; de Klein and van Logtestijn 1996; Weier et al. 1993; Ryden and Lund 1980) have emphasized the importance of soil or substrate water content on denitrification. It was observed that evolution of gaseous N emissions depended on the exceedance of a threshold water content, below which denitrification was apparently inhibited by presence of oxygen. Yet, threshold values of water content usually are mean values which do not consider gradients of water content within the investigated soil or substrate volume.

The inhomogeneous distribution of water in potted and planted substrate is confirmed by the presented data (Fig. 1). As pots of *P. zonale* were irrigated by flooding, the water content of the substrate was highest in the lowest layer at the pot bottom and decreased with height. The difference in water content between the substrate layers was especially high shortly after irrigation. With time, water content in all layers decreased and differences between them grew smaller, which was obviously due to plant water uptake. Accordingly, the difference in water content between the lower layer and the mean of the whole substrate core decreased with decreasing mean water content (Fig.2).

The decrease of substrate water content with increasing distance from the pot bottom was described by Fonteno (1989).

So, at least for a certain time period after irrigation, the lower substrate layers of flood irrigated pots proved to possess the highest water content. Thus, it was suggested that these layers were mostly responsible for N_2O emissions from potted substrate. The appropriateness of this assumption was confirmed by measurement of redox potential.

7.4.2 Denitrification and redox potential in planted peat substrate following irrigation

The redox potential results from the biochemical (microbial) reduction processes that take place in a soil or substrate. During anaerobiosis, several oxidation-reduction systems are reduced which under aerobic conditions would be present in the oxidized form (Patrick and Mahapatra 1968). Once oxygen is consumed, e.g. during waterlogging of a soil, the redox potential decreases and nitrate is reduced as it is used next for microbial electron transfer (nitrate respiration).

This could be well observed during simultaneous measurement of denitrification and redox potential in pots of *P. zonale* (Fig. 4). The course of N emissions was in many cases reflected by changes in redox potential. It was mostly the electrodes, that were placed close to the pot bottom, which indicated a decrease of redox potential at the same time as N emissions increased. While at the onset of denitrification there was high synchrony between decline of redox potential and rise of N emissions, redox potential tended to increase sooner than N emissions decreased. It is assumed that this resulted from increasing diffusion of air (and oxygen) into the substrate, which generally raised the redox potential but still allowed existence of denitrifying sites, which were not all reflected by the few electrodes that were applied. The observed synchrony of redox potential and N emissions at the start of the experiment indicates that time lags in the emission of N gas from the irrigated substrate did not occur. Delayed release of N emissions from soil has been reported in literature and was considered a possible hazard to denitrification measurement as it may lead to underestimation of total N gas production (McCarty et al. 1999; Clough et al. 2000). Presumably, the high porosity of the peat allowed escape of N gases from the substrate inspite of high water content.

Although development of N emissions and redox potential were generally in accordance, the amount of electrodes per pot indicating a change in redox potential as well as their amplitude were highly variable and did not necessarily correspond to the height of N emissions (Fig. 4). This hints at inhomogeneity of reducing conditions inspite of the very limited substrate volume (340 mL). Even in the lower substrate layers there were electrodes that did not show changes in redox potential, although all electrodes that indicated a change were located close to the pot bottom. Possibly this can be related to observations made by Sexstone et al. (1985b), who

investigated oxygen profiles and denitrification in soil aggregates. They reported that denitrifying activity was restricted to aggregates with anaerobic centers, but not all aggregates with anaerobic zones denitrified. This they related to a possible lack of carbon or nitrate. Also in the presented study, the missing decrease of redox potential at some electrodes might be due to a local lack of carbon. As easily decomposable carbon is the source of electrons for microbial metabolism, its availability is a premise for reduction processes. In addition, availability of carbon increases microbial activity overall and thus, accelerates oxygen depletion. So, the amount of locally available carbon may be responsible for variability of redox potential. It might be related to plant roots, as indicated by literature.

Decreasing redox potential has been observed at the root tip of various plant species cultivated in aerobic soils (Flessa and Fischer 1992; Fischer et al. 1989). This effect was attributed to oxygen uptake and release of root exudates, which have reducing properties or may serve as substrates for microorganisms. Also, decreasing redox potential was found in the proximity of dead roots (Fischer et al. 1989; Fischer and Schaller 1980) and close to incorporated sugar beet residues (Flessa and Beese 1995). In all these cases, the decline of redox potential was locally very limited and the effect on redox potential was nullified within few millimeters of distance. In accordance with this, Parkin (1987) traced back 85 % of the denitrification activity of a 98 g soil core to 0.08 g of soil containing a piece of decaying plant material. The positive effect of carbon supply on denitrification was also observed in previous studies with potted plants (Chapter 5), which indicated that in this growing system denitrification was limited by carbon availability.

Thus, the observed variability of redox potential in pots of *P. zonale* might result from growth (root tips) and decay of plant roots in combination with inhibited oxygen diffusion due to high water content. The electrodes per pot that indicated a decrease of redox potential were presumably positioned by chance at sites of high reducing potential. With regard to the origin of gaseous N emissions, the presented study confirmed that in the applied growing system the biggest part of denitrification N loss from potted substrate originated from the lower substrate layers.

8. Horticultural practice and denitrification

8.1 Introduction

In previous studies with cultures of potted ornamental plants denitrification in planted substrate proved to be mainly limited by substrate water content, which decreased after irrigation because of evapotranspiration (Chapter 4). Yet, it was suggested that the rate of decrease might also be influenced by the volume of water per plant, which was assumed to depend on irrigation practice, substrate volume, and pot type.

Irrigation practice was considered likely to influence substrate water content, e.g. by the duration of flooding. While short irrigation periods of 15 to 40 minutes were recommended in literature to avoid waterlogging and root damage (Steffen 1989, Strauch 1989), longer time periods were reported to be common in horticultural production (Steffen 1989).

Also, pot design was estimated to influence denitrification. There are various pot types available on the market which differ e.g. in size and amount of pot bottom holes. The pot size determines the substrate volume per plant and thus also the amount of water available for evapotranspiration. Pot bottom holes were considered to affect water uptake and drainage of substrate. As the substrate layer close to the pot bottom was the main source of denitrification N emissions (Chapter 7), it was suggested that pot bottom holes might also influence denitrification by affecting air exchange between denitrifying substrate and atmosphere.

As the speed of decrease of substrate water content proved to be influenced by variable climate factors (temperature, vapour pressure deficit (vpd)) which change during day, it was assumed that the daytime of irrigation might affect denitrification. In unplanted substrate increase of temperature showed a clear positive effect on N emissions (Chapter 3). Yet, in planted substrate increase of vpd, which usually coincided with increase of temperature, reduced gaseous N loss by decreasing substrate water content through plant transpiration (Chapter 4). Thus, it was decided to investigate the effect of daytime of irrigation on denitrification N loss.

So far, denitrification measurement had focused on potted and planted substrate. Yet, it was questioned if denitrification also took place on surfaces used for cultivation

of ornamental plants. As denitrification requires humidity (lack of oxygen) and availability of nitrate and carbon, it was suggested that denitrifying activity could develop in any place that offered these conditions, e.g. in irrigation mats.
To prove all these assumptions the following treatments were analyzed with regard to denitrification N loss:

1. Irrigation period of 2 hours and of 0.5 hours,
2. Pot volume of 240 mL and of 550 mL,
3. Four pot types differing in amount and size of bottom holes,
4. Irrigation start in the morning (9 a.m.) and in the afternoon (3 p.m.),
5. Incubation of irrigation mat used with and without plastic film cover.

8.2 Materials and Methods

8.2.1 Experimental setup for denitrification measurement

Flow-through chambers like described in Chapter 2.2.2 were used for measurement of denitrification from planted substrate. Only for measurement of N emissions from irrigation mats closed glass jars were used (Chapter 2.2.2).
Before the start of the experiment by flood irrigation substrates and mats were pretreated with acetylene (C_2H_2) within chambers or jars as described in Chapter 8.2.2.
The irrigation event was defined start of the experiment. Air samples were taken from chambers with syringes topped with gauge needles from silicon tubes at the air outlet every two to four hours until N_2O emissions ceased. Closed jars were sampled with syringes after 48 hours of incubation.

8.2.2 Application of C_2H_2

For determination of (N_2+N_2O)-N emission by denitrification, 5 vol.% C_2H_2 was added to the chamber and jar atmosphere. To guarantee immediate inhibition of N_2O reduction to N_2 substrates and mats were pretreated for two hours with 5 vol.% C_2H_2 prior to irrigation.

8.2.3 Duration of fertigation and composition of fertigation solution

For denitrification measurement, pots were fertigated with 1 L of a solution containing 150 mg NO_3 -N L^{-1}. At the end of irrigation the fertigation solution was released by an opening at the bottom of the chambers. Generally flood irrigation lasted two hours, only in one experiment it was varied from two to 0.5 hours.
Pieces of irrigation mat were wettened with 10 mL of the above solution prior to incubation.
During cultivation plants were fertigated with 1g L^{-1} of a full compound fertilizer (Flory3, Euflor) according to horticultural practice.

8.2.4 Substrates

Commercial, fertilized white peat substrates were used for planting.

8.2.5 Plant material

Experiments were conducted with plants of *Pelargonium zonale* 'Grand Prix'. Plants were propagated by cuttings and rooted in small peat nuggets (Jiffy7) for two weeks. Then plants were cultivated for at least four weeks to guarantee rooting of the substrate. For each experiment plants of the same set were used, i.e. plants were of the same age and of similar growth.

8.2.6 Pot types

Plants were potted into 240 mL, 340 mL, 550 mL, or 650 mL plastic pots. If not mentioned differently, 340 mL pots of uniform dimensions were used. Pots of 650 mL volume varied in size and amount of bottom holes (Fig. 4).

8.2.7 Irrigation mat

Commercial irrigation mat (fleece material) topped with or without black plastic film was used for denitrification experiments.

8.2.8 Analytical procedures

8.2.8.1 N_2O

The analysis of N_2O in all air samples was performed by a gas chromatograph (Chrompack 9001) with an electron capture detector (ECD) according to a method described by Mosier and Mack (1980).

8.2.8.2 Water content

During denitrification measurement every four to eight hours pots were taken from the chambers and weighed. At the end of the experiment plants were harvested, the fresh weight of the shoot and of the rooted substrate was determined. Then, the substrate was dried at 105°C and weighed again. Water content (vol.%) was calculated from pot volume, fresh weight of shoot, dry weight of rooted substrate, and fresh weight during the experiment.
The water content of unplanted potted substrate was determined by weighing the substrate before and after drying at 105°C .

8.2.8.3 Vapour pressure deficit (vpd)

Temperature and relative humidity were determined during denitrification measurements. Both values were used to calculate the vapour pressure deficit (Murray 1967, Malberg 2002):

$$vpd = e°(T) - e$$

$$e°(T) = 0.6108\exp[17.27*T/(T+237.1)]$$

$$e = e°(T) * rh/100$$

vpd = vapour pressure deficit [kPa]
e°(T) = saturation vapour pressure [kPa]
T = Temperature [°C]
e = vapour pressure [kPa]
rh = relative humidity [%]

8.2.8.4 Statistics

Statistics were performed by use of the SAS package. Denitrification measurements were conducted with six replications per treatment.

8.3 Results

8.3.1 Duration of flood irrigation

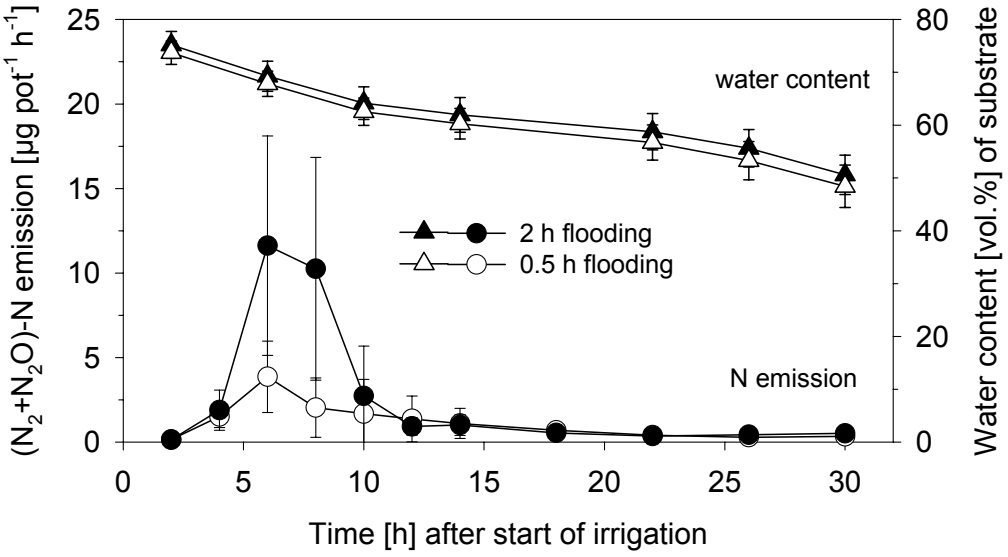
8.3.1.1 Effect of irrigation duration on denitrification

When *P. zonale* plants grown in white peat were flood irrigated for 0.5 hours and for 2 hours, respectively, it resulted that the 2 hour irrigated pots emitted higher amounts of (N_2+N_2O)-N (Fig. 1). After 0.5 hours of flood irrigation, (N_2+N_2O)-N emissions were only half as high as after 2 hours of flood irrigation (Tab. 1). N_2O emissions of the short irrigation period amounted only to 1/3 of the longer irrigation time. Thus, the ratio N_2O-N:N_2 was higher in the 2 hour irrigation treatment.

While N loss was strongly affected by irrigation time, the mean substrate water content differed only slightly. The 0.5 hour treatment showed an on average 1.5 vol.% lower mean water content than the 2 hour treatment during the experiment.

For further investigation, water distribution in unplanted substrate was examined after 0.5 hours and 2 hours of irrigation.

Fig. 1 (N_2+N_2O)-N emissions and water content of a white peat substrate planted with *P. zonale* after 2 h and 0.5 h of flood irrigation (5 vol.% C_2H_2)



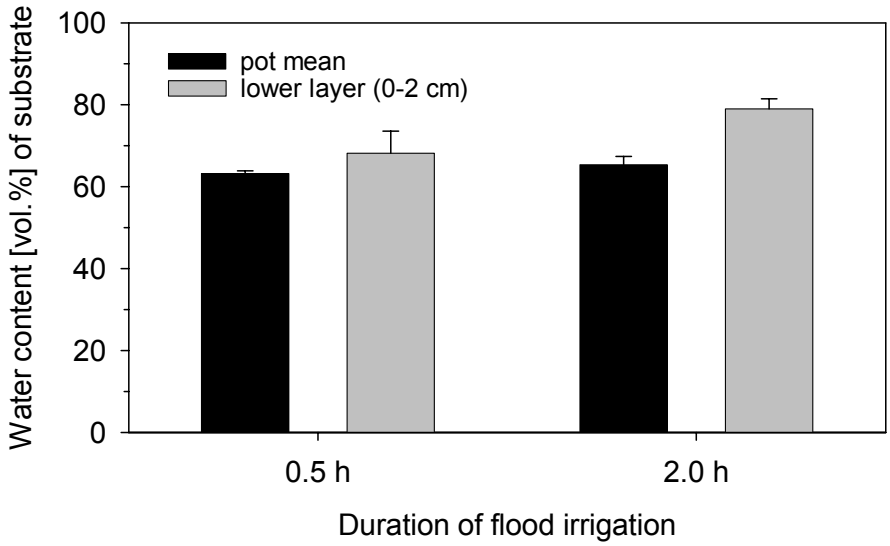
Tab. 1 Sum of (N_2+N_2O)-N and N_2O-N emissions from pots of *P. zonale* planted in white peat after 2 h and 0.5 h of flood irrigation (+/-5 vol.% C_2H_2)

Duration of flood irrigation	(N_2+N_2O)-N (µg pot^{-1})	N_2O-N (µg pot^{-1})	ratio N_2O-N/N_2
0.5 h	29 ± 20	5 ± 2	0.19
2 h	62 ± 24	14 ± 4	0.28

8.3.1.2 Effect of irrigation duration on water distribution in potted peat substrate

Like in denitrification measurement, mean substrate water content differed only by about 2 vol.% when potted and planted peat substrate was flood irrigated for 0.5 h and 2 h, respectively (Fig. 2). Yet, water content of the substrate layer at the pot bottom was more than 10 vol.% higher after 2 hours than after 0.5 hours of flooding. As this substrate layer had a relatively small volume (70 mL), its change in water content was hardly reflected in mean water content.

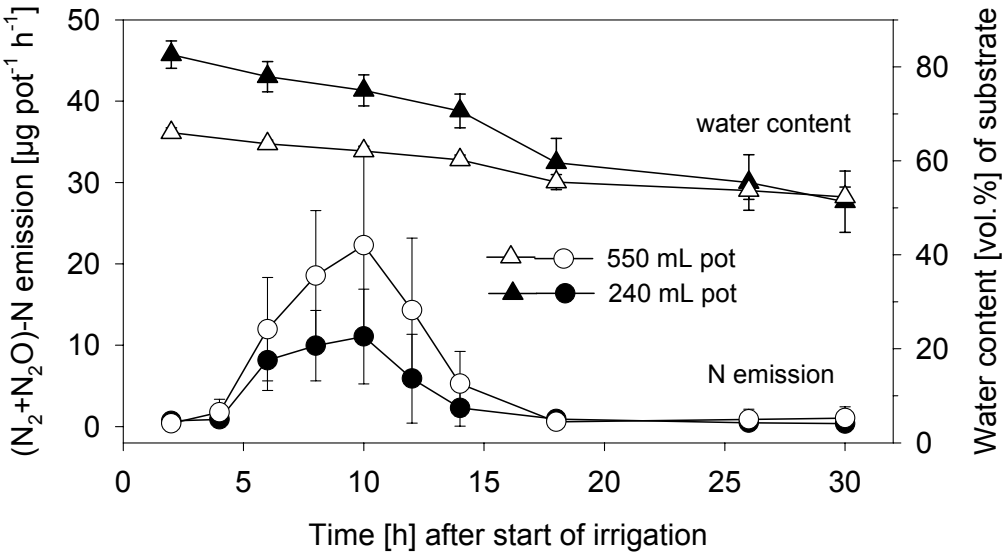
Fig. 2 Water content of substrate layers after 0.5 hours and 2 hours of flood irrigation of potted peat substrate planted with *P. zonale*



8.3.2 Effect of pot size on denitrification

The pot size determines the substrate volume per plant. To investigate its influence on denitrification, *P. zonale* were planted in 240 mL and 550 mL plastic pots. When denitrification N loss was measured, it resulted that N emissions from 550 mL pots were higher and lasted about four hours longer than from 240 mL pots (Fig. 3). In total, 550 mL pots emitted twice as much (N_2+N_2O)-N as 240 mL pots (Tab. 2). Production of N_2O-N increased by nearly 60 % when 550 mL instead of 240 mL pots were used. Thus, it seemed that a higher substrate volume per plant lead to increased denitrification N loss.

Fig. 3 (N_2+N_2O)-N emissions and water content of a white peat substrate planted with *P. zonale* as affected by pot size (240 mL vs. 550 mL)



Tab. 2 (N_2+N_2O)-N and N_2O-N emissions of a white peat substrate planted with *P. zonale* as affected by pot size (240 mL vs. 550 mL) (+/- 5 vol.% C_2H_2)

	N loss per pot	
Pot volume	(N_2+N_2O)-N [µg]	N_2O-N [µg]
240 mL	92 ± 41 *a**	67 ± 36 a*
550 mL	181 ± 88 *b**	108 ± 40 a*

*Different letters indicate statistically significant differences between treatments (α =0.05, t-Test)

8.3.3 Effect of pot type on denitrification

To investigate the effect of pot design on denitrification, plastic pots with different numbers of bottom holes were used for denitrification measurement (Fig. 4). All pots except type 2 were of the same dimensions. Type 2 had a higher diameter (+8 mm) at the pot bottom and thus a more cylindrical form than the other models. Substrate water content after irrigation and also during denitrification measurement was not affected by pot type relative to the control. When denitrification N loss depending on pot type was determined, emissions from pot type 1 were nearly twice as high as those from the control pot (Fig. 5). Pot type 2 produced more than 40 % higher N loss than the control, and only pot type 3 showed lower N loss than the control (- 15 %). Because of high variability of N loss per pot, differences between all pot types and the control treatment were statistically non-significant (t-Test, α =0.05).

Fig.4 Bottom design and diameter of pots used for denitrification measurement

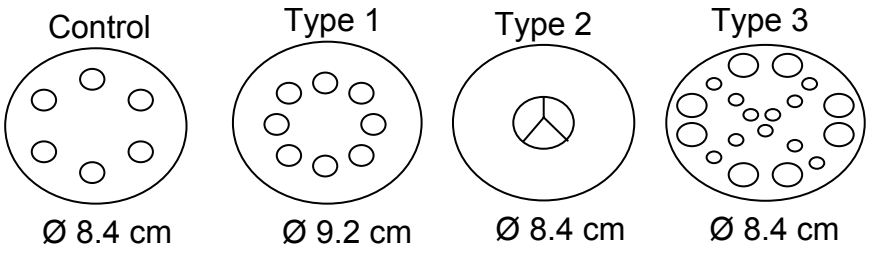
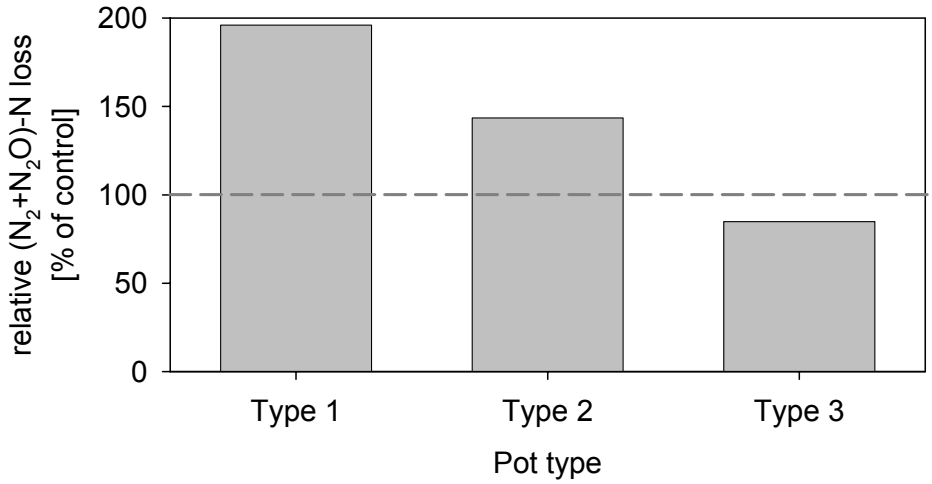


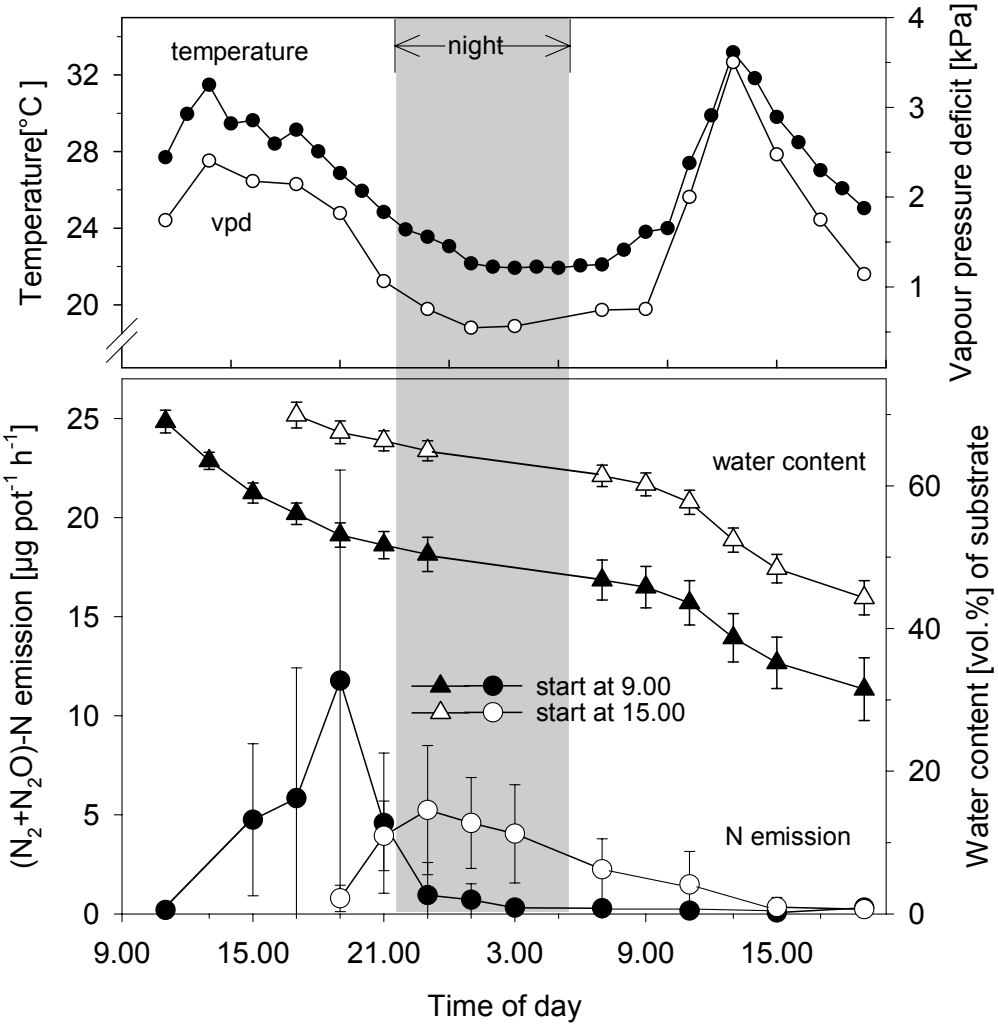
Fig. 5 Relative (N_2+N_2O)-N loss after irrigation from pots of *P. zonale* planted in peat substrate as affected by pot type (5 vol.% C_2H_2)



8.3.4 Time of day of irrigation

When pots of *P. zonale* planted in white peat were irrigated for two hours at 9 a.m. and at 3 p.m., respectively, the course of N-emissions varied between treatments (Fig. 6). The pots that were irrigated in the morning showed a high maximum, and then a sharp decline of emissions in the afternoon. In contrast, N emissions from pots of the later irrigation time proceeded more evenly. They were comparatively lower, but lastet for a longer time relativ to the 9 a.m. treatment.

Fig. 6 (N_2+N_2O)-N emissions and water content of a white peat substrate planted with *P. zonale* after 2 h of flood irrigation starting at 9 a.m. and 3 p.m. (5 vol.% C_2H_2) and greenhouse temperature and vapour pressure deficit (vpd) during measurement



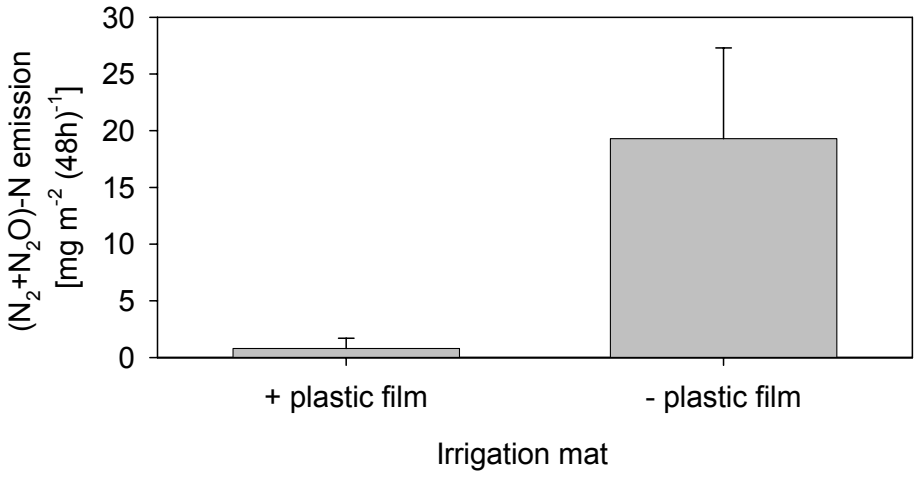
Simultaneously, temperature and vapour pressure deficit (vpd) were high at midday and in the afternoon. Both decreased in the evening and maintained relatively constant values during night.

Inspite of differences in the course of N emissions, pots from both treatments emitted about the same amount of N: 64 µg pot^{-1} in the 9 a.m. treatment, 60 µg in the 3 p.m. treatment. So, the high N emissions of the 9 a.m. treatment were apparently compensated for by the long duration of emissions in the 3 p.m. treatment.

8.3.5. Denitrification N loss from irrigation mat

To investigate gaseous N loss from horticultural production system beside potted substrate, irrigation mats that were used for the cultivation of potted plants with or without protection by plastic film were incubated for denitrification measurement. After 48 hours of incubation the mat without plastic film had emitted nearly 20 mg N m^{-2}, while the mat that was covered with plastic film emitted only about 1 mg N m^{-2} (Fig. 7). During cultivation of plants the unprotected mat was directly in contact with pots and the atmosphere. Thus, it was exposed to sunlight, spilled substrate and plant material.

Fig. 7 (N_2+N_2O)-N emissions from irrigation mat with or without plastic film after 48 hours of incubation (5 vol.% C_2H_2)



8.4 Discussion

8.4.1 Duration of flood irrigation

Denitrification N loss from potted peat substrate planted with *P. zonale* was reduced by half when irrigation time was shortened from 2 hours to 30 minutes (Tab. 1). The time length of N emissions was similar in both treatments, but pots of the 2 hour treatment showed much higher emission rates than those of the 0.5 hour treatment (Fig. 1). The big difference in emission rates and N loss was surprising because mean substrate water content was only about 1.5 vol.% higher after 2 hours of irrigation. However, the difference in water content of the lower substrate layer (0-2 cm from pot bottom) was more than 10 vol.% higher in the 2 h treatment (Fig. 2). Thus, the higher N loss of the 2 h treatment might be due to this difference in water content, since the lower substrate layer proved to be the source of most of the denitrification N emissions per pot (Chapter 7).

For horticultural practice the duration of flood irrigation is recommended to be between 15 to 40 minutes (Steffen 1989, Strauch 1989). Short and frequent flooding is considered to avoid root damage by water logging of the lowest substrate layer and also to prevent drying of substrate beyond rewettability (Strauch 1989). Yet, realization of short flooding periods depends on technical equipment, e.g. high flow rates of valves for quick filling of irrigation tables and high volume drains for rapid remove of irrigation water. The applied irrigation time in horticultural practice, including flooding and draining, was estimated to be one hour or more (Steffen 1989).

With regard to denitrification, short irrigation periods seem favorable to reduce gaseous N loss and oxygen deficiency within the substrate. In the presented study difference in water content was much smaller than difference in N loss, thus a mitigating effect on N emissions may be expected even if irrigation frequency was slightly increased to compensate for lower water content after irrigation.

8.4.2 Effect of pot size on denitrification

(N_2+N_2O)-N emissions from 550 mL pots were higher and lasted about four hours longer than from 240 mL pots (Fig. 3). Summed up (N_2+N_2O)-N loss from 550 mL

pots planted with *P. zonale* was twice as high as from 240 mL pots (Tab. 2). Also N_2O production was by 40 % higher in 550 mL than in 240 mL pots. Plant size did not differ between treatments. Yet, it may be assumed that doubling of N emissions from 550 mL pots relative to 240 mL pots was caused by nearly doubling of the substrate volume of the 0-2cm layer at the pot bottom. The volume of this substrate layer amounted to 64 mL in the smaller pots and 103 mL in the bigger pots. In previous studies, the lower substrate layer was found to be mostly responsible for denitrifying activity in potted and flood irrigated substrate (Chapter 7).

In the presented study, pot size significantly increased N loss per irrigation event. Still, it was questioned if this difference in N loss persisted over the cultivation period. Although 550 mL pots showed lower water content [vol.%] after irrigation (Fig. 3), they contained more water [mL] because of higher volume and thus, they were less frequently irrigated than smaller pots. When calculations considered irrigation frequency as well as N loss per irrigation event, then there was no more significant difference between 240 mL and 550 mL pots (Tab. 3). The higher N loss of the bigger pots per irrigation event was compensated for by less frequent irrigations.

Tab. 3 Estimation of (N_2+N_2O)-N loss from 240 mL and 550 mL pots considering N loss per irrigation event and irrigation frequency

	240 mL pot	550 mL pot
Water content of substrate after irrigation [vol.%]	76	69
Threshold water content for irrigation [vol.%]	50	50
Water content of substrate after irrigation [mL pot^{-1}]	182	380
Threshold water content for irrigation [mL pot^{-1}]	120	225
Delta /difference [mL]	62	155
Evapotranspiration [ml h^{-1} plant $^{-1}$]	1.8	1.8
Time until threshold for irrigation is reached [h]	34.4	86.1
N loss [µg $(irrigation\ event)^{-1}$]	92	181
Rate of N loss [µg h^{-1}] during irrigation cycle	2.7 a*	2.1 a*

*Similar letters indicate statistically non-significant differences (α =0.05, t-Test)

8.4.3 Effect of pot type on denitrification

Denitrification in horticultural substrates depends on oxygen deficiency (Chapter 4). Consequently, it is restricted by factors that promote air exchange and oxygen diffusion into the substrate. The amount of pot bottom holes was considered to influence substrate water content, air exchange and the availability of oxygen at the very site of denitrification close to the pot bottom (Chapter 7).

When pots with different sizes and numbers of bottom holes (Fig. 4) were used for denitrification measurement, substrate water content after irrigation did not differ between pot type 1, 2, 3, and the control treatment. Thus, number and size of pot bottom holes did not affect substrate water uptake. When pots were compared to the control pot type with regard to denitrification N loss, the influence of size and amount of bottom holes seemed to be rather low (Fig. 5). Only pot type 1 tended to produce higher N loss than the control treatment. As this pot type had two more bottom holes than the control pot, the amount of holes could not be responsible for the higher N loss. Further calculations revealed that the substrate volume of the 0-2 cm layer, in which most of the denitrifying activity took place (Chapter 7), was nearly one third higher in pot type 1 (150 mL) than in the control pot (115 mL). This difference in volume resulted from an 8 mm higher diameter at the pot bottom of type 1 relative to the control (Fig. 5). Pot type 2, which had only one centered bottom hole, produced about 40 % more N emissions than the control, while pot type 3 with its multi-hole bottom emitted 15 % less N than the control. In all cases, difference to the control treatment was statistically non-significant because of high variability of N loss per pot. Apparently, denitrification was more influenced by the shape of pots and by the resulting volume of the lower substrate layer than by the number of pot bottom holes.

8.4.4 Time of day of irrigation

The sum of denitrification N loss per pot was about the same wether pots were irrigated in the morning (9 a.m.) or in the afternoon (3 p.m.) (Chapter 8.3.4). Yet, the course of N emissions varied between treatments (Fig. 6). Emissions from the early irrigated pots reached a high maximum 10 hours after irrigation and then rapidly declined within four hours towards zero. The late irrigated pots emitted N at relatively low rates, but over a longer period of 20 hours. The difference in the run of the

curves might be ascribed to climate factors. While high temperature around midday was likely to promote denitrification in early irrigated pots, the concurrently high vapour pressure deficit (vpd) was presumably responsible for a fast decrease of substrate water content. Thus, N emissions rose high and ceased rapidly. When denitrification started in the late irrigated pots, temperature declined soon and presumably it did not stimulate denitrification exceedingly. At the same time, vpd declined too, so substrate water content hardly decreased. Consequently, N emissions were not very high under these relatively stable conditions, but they lasted over a long time period.

Stimulation of denitrifying activity by increasing temperature is well known in literature (Stanford et al. 1975, Smid and Beauchamp 1976, Aulakh et al. 1992, Dobbie and Smith 2001). Yet, most investigations focused on unplanted soils incubated in closed assay systems. The promoting effect of temperature on denitrification was also observed in previous studies of unplanted peat substrate incubated in closed jars (Chapter 3). There, the positive effect of increasing temperature on evolution of N gases was very clear, while in experiments with planted substrate the stimulating effect of temperature on denitrification was overlaid by the more dominant effect of substrate water content. The presented results hint at the ambiguity of temperature with regard to denitrification. On one hand, temperature stimulates denitrification by increasing overall microbial activity and accelerating oxygen consumption. On the other hand, high temperature shortens the duration of denitrifying activity by increasing vpd and thus, accelerating the decrease of substrate water content.

8.4.5. Denitrification N loss from irrigation mat

During denitrification measurement only N loss was recorded that originated from potted substrate. Yet, it was questioned if gaseous N emissions may also develop on surfaces used for production of potted ornamental plants. As irrigation mats are in close contact with potted plants and as they are still common in horticultural production, they were used for incubation studies.

Denitrification N loss from irrigation mat depended strongly on wether it was used in combination with protective plastic film or not (Fig. 7). With plastic film N loss was comparatively low and hardly reached 1 mg N m^{-2} in 48 hours. Without plastic film N loss was more than 20 times higher and amounted to 19.3 mg N m^{-2} in 48 hours.

Presumably, this extremely high N loss resulted from increased microbial activity due to contamination of the unprotected mat with substrate and plant material (plant roots, algae growth). As the irrigation mat was kept humid during cultivation of plants and NO_3 was frequently supplied by fertigation, conditions were likely to be favorable for microbial denitrification.

These observations indicate that occurrence of humidity in combination with availability of nitrate and carbon anywhere in the cultivation system may be a source of gaseous N emissions.

9. Discussion

9.1 Evaluation of denitrification N loss from potted ornamental plants

9.1.1 Dimensions of denitrification N loss

Mean N emissions from horticultural pot plant production were calculated using data of all denitrification measurements (control treatments) conducted within this study (Tab. 1). The absolute amounts of (N_2+N_2O)-N and N_2O-N emissions calculated per hectare were based on the assumption of year-round production, a mean plant density of 24 plants m^{-2} (Rothenburger 1996), and three irrigation events per week (flood irrigation). Estimates are valid for cultivation in closed irrigation system only and do not include potential emissions from surfaces used for cultivation. Yet, it should be mentioned that open irrigation systems still exist in german horticulture as indicated by a survey of ZMP (2000).

In comparison to (N_2+N_2O)-N loss from agricultural soils, emissions from horticultural pot plant production were rather low and only amounted to about 30 % of agricultural emissions (Tab. 1). Yet, the potential for denitrification in horticultural substrates was high. When calculations were based on soil volume per hectare considering a soil depth of 30 cm (3000 m^3 soil), (N_2+N_2O)-N emissions from horticultural substrate rose to 191 kg $year^{-1}$. Thus, the comparatively low N loss was due to the small substrate volume per surface area in pot plant production.

N_2O-N loss, in contrast, was comparable in both production systems. Apparently, a higher share of the environmentally harmful gas was emitted in horticultural than in agricultural production. Causes for this might be the high porosity of horticultural substrates which was considered to allow quick escape of gases from denitrifying sites (Chapter 7), and thus reduce the probability of further N_2O reduction. Additionally, conditions in planted substrate often might not be totally anaerobic during denitrification, e.g. because of high substrate air capacity (Chapter 6) and quick changes of substrate water content after irrigation (Chapter 4). Imperfect

anaerobiosis has been reported to favour emission of N_2O relative to N_2 (Firestone et al. 1979, Mosier et al. 1998, Aulakh et al. 1992).
The low relative N losses (N loss : N applied) from horticultural production likely resulted from the intensive application of fertilizer N in this growing system.

Tab. 1 Average (N_2+N_2O)-N and N_2O-N emissions due to denitrification from pot plant production as estimated from data of the presented study and from agricultural soils in temperate climate as reported in literature

	Emissions [kg ha^{-1} $year^{-1}$]		Emission [% of applied N]	
	(N_2+N_2O)-N	N_2O-N	(N_2+N_2O)-N	N_2O-N
Hort. pot plant production	6.9	2.4	1.2	0.4
Agricultural soils	20 – 30 *	1 - 3 **	ca. 10 *	0.8 – 1.5 **

* source of data: Nieder et al. 1989; von Rheinbaben 1990

** source of data: Bouwman and Boumans 2002; Beauchamp 1997; Kaiser and Ruser 2000; Skiba et al. 1996; Kaiser and Heinemeyer 1996; Mosier et al. 1996; Goossens et al. 2001

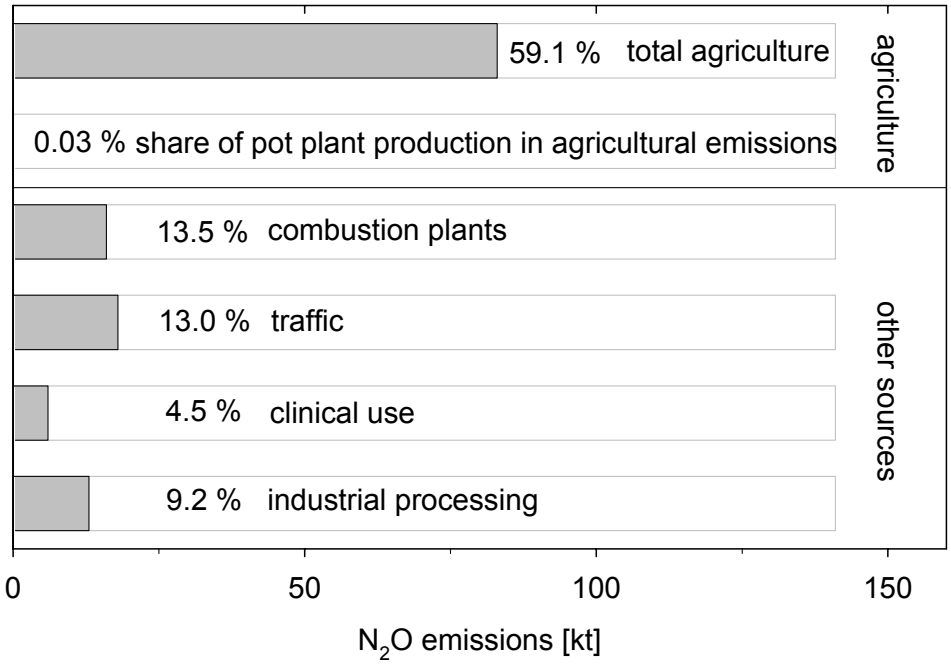
9.1.2 Evaluation of denitrification N loss from the economic point of view

According to the above estimate (Tab. 1) about 7 kg of N fertilizer per ha and year are lost from pot plant production. Balancing this loss of nitrogen by use of a common soluble full compound fertilizer would cause additional costs of about 50 Euro per ha and year. If N loss was compensated for by a soluble single compound fertilizer containing only nitrogen, then monetary loss would be reduced to about 18 Euro per ha and year.
These additional costs due to N loss by denitrification are low and thus insignificant to pot plant producers from the economic point of view. This is confirmed by estimates based on calculations of Rothenburger (1996) which revealed that in pot plant production fertilizer costs hardly account for 1 % of total production costs.
Still, multiplied by the area used for pot plant cultivation in Germany, denitrification N loss is estimated to amount to 38.7 tons per year.

9.1.3 Evaluation of denitrification N loss from the ecologic point of view

The average share of N_2O in (N_2+N_2O)-N emissions from pot plant cultivation as determined in this study amounted to 35 % (Tab. 1). So, relative N_2O emission was much higher from horticultural substrate than from agricultural soils. Because of the comparably low production area, the absolute amount of N_2O emitted from pot plant production in Germany only amounted to 21.4 t N_2O $year^{-1}$, which corresponds to 0.03 % of estimated N_2O emissions from agriculture (Fig. 1). Thus, the contribution of pot plant cultivation to total N_2O emission in Germany is considered to be low.

Fig. 1 Sources of N_2O emissions in Germany as estimated by UBA (2000), supplemented by estimated values for pot plant production



9.2 Dynamics of denitrification and the effect of horticultural cultivation practice

As discussed above (Chapter 9.1.2, 9.1.3), economic loss due to denitrification in horticultural pot plant production was estimated to be low, and so was the contribution of pot plant production to total N_2O emission in Germany. Yet, studies showed that denitrification N emissions can vary strongly from the presented estimates due to variation of physical substrate properties (Chapter 6), or because of differences in horticultural practice (Chapter 8). Additionally, surfaces used for plant production may contribute considerably to N emissions. Studies indicated that denitrification may occur in any place that offered high moisture (low oxygen) as well as availability of nitrate (fertilizer) and carbon (e.g. plant residues). Especially the use of open irrigation systems leading to wetting of larger areas is considered to lead to considerable increase of estimated values.

To keep denitrification at a low level, understanding its dynamics as well as the influence of horticultural management is necessary. Thus, the essence of the presented study regarding influencing factors of denitrification, sources of variability of N emissions, and possibilities to minimize denitrification by cultivation practice is presented in the following.

9.2.1 Factors influencing denitrification in cultures of potted ornamental plants and sources of their variability

9.2.1.1 Substrate water content and oxygen availability

Water content proved to be the most dominant and also the most dynamic factor controlling denitrification (Fig. 2). Being the counterpart of air content, its effect on denitrification was attributed to restriction or permittance of oxygen diffusion into the substrate. Evolution of N gases could only be observed at high water content, corresponding to low air content, after irrigation. Thus, substrates of high air capacity and of high stability during cultivation were favorable for restricting denitrification by maintaining high substrate air content after irrigation events (Chapter 6). In contrast, stimulation of denitrification occured by reduction of air capacity, e.g. due to increased bulk density by compaction or shrinkage of substrate during cultivation

(Chapter 6). Similarly, substrate air content decreased and N emissions rose with increasing water volume retained by the substrate after irrigation. Higher water content after irrigation was reached with increasing duration of flooding (Chapter 4, 8) and with decreasing dryness of substrate before irrigation (Chapter 4, 6). Thus, adjusting frequency and duration of flood irrigation may serve to raise substrate air content and to limit denitrification.

After flood irrigation substrate air content was lowest close to the pot bottom. Consequently, the lower substrate layer was the main source of N emissions (Chapter 7). Its volume changed with pot size and pot shape and affected the quantity of N emissions. Lower pot volume and smaller diameter at the pot bottom decreased the volume of denitrifying substrate as well as N emissions per irrigation event (Chapter 8). Thus, the choice of pot type may restrict denitrification unless reduction of N loss per irrigation event was compensated for by increased irrigation frequency.

The decline and ending of N emissions from horticultural substrate began as soon as water content dropped below a threshold value (Chapter 4, 6). Yet, because of variability and dependance on substrate properties no universal threshold value of mean substrate water content for denitrification could be elaborated (Chapter 6). The decrease of substrate water content was driven by evapotranspiration, which depended on climate (vapour pressure deficit (vpd)) and on plant size (Chapter 4, 5). High N emissions could be related to low vpd causing low evapotranspiration, slow decrease of substrate water content, and long duration of denitrifying activity (Chapter 4). Thus, control of temperature and air humidity, which determine vpd, may allow to accelerate reaching of the threshold value for denitrification and thus, to reduce N emissions.

In the presented study, like in commercial pot plant production, substrate and available water volume per plant were rather small. Consequently, substrate water content was in general sufficiently reduced within 20 to 34 hours after irrigation for denitrification to stop. Thus, within comparatively little time following each irrigation cycle the rise and decline of N emissions took place.

9.2.1.2 Carbon availability

Carbon availability proved to be the next important factor controlling denitrification following substrate water content (Fig. 2). In unplanted peat substrate denitrification

N loss was consistently increased by addition of rising concentrations of glucose-C (Chapter 3). This indicated that supply of easily available carbon was generally low in peat substrate and that there, denitrification was limited over a broad range by carbon deficiency. Similarly, supply of glucose-C increased denitrification N loss in planted substrate (Chapter 5). Apparently, the limitation persisted inspite of the presence of plants, although plants were known to serve as carbon suppliers to soil or substrate (von Rheinbaben and Trolldenier 1984).
Limitation of denitrification by low carbon availability may be fortified by use of stable substrates of low biodegradability and by avoidance of waterlogging of plant roots as oxygen deficiency was reported to stimulate root exudation (Wollersheim et al. 1987).

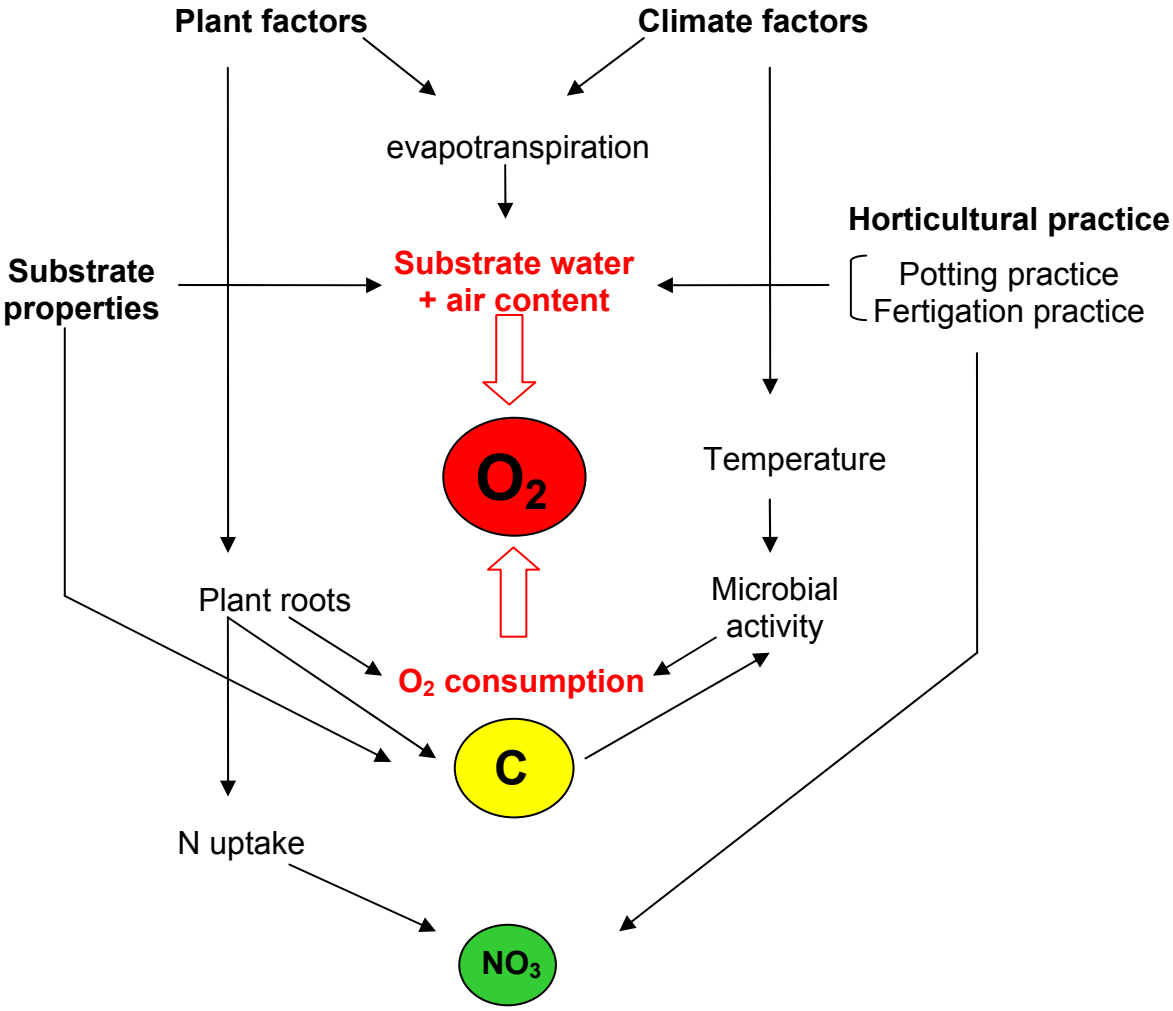
9.2.1.3 Nitrate availability

Only in unfertilized peat substrate denitrification was limited by nitrate availability (Chapter 3). Even the lowest fertilization level allowed 'maximum' (N_2+N_2O)-N loss, which was not increased by further NO_3 supply. In contrast to (N_2+N_2O)-N emissions, the share of N_2O emissions did increase with rising NO_3 supply.
Thus, neither reduction nor stimulation of (N_2+N_2O)-N loss was likely to occur in horticultural growing systems where substrate nitrate content was permanently resupplied by fertigation. But, high NO_3 fertilization could be expected to increase the emission of environmentally harmful N_2O.

9.2.1.4 Sources of variablility

High spatial and temporal variability of N emissions is characteristic for denitrification in soil (Folorunso and Rolston 1984, Christensen et al. 1990b). The same may be stated for horticultural substrates. At first sight, this seemed surprising because in comparison to natural soils, horticultural white peat substrates were considered to be rather homogeneous regarding both, physical and chemical properties. In accordance with this assumption, variability of denitrification N loss in unplanted substrate was relatively low (Chapter 2). Still, whenever planted substrate was investigated variability of N emissions was high. In fact, several causes for variability of N loss could be related to plant factors and to their influence on denitrifying conditions (see also Fig. 2). All sources of variability that were discovered in the presented study are summarized in the following.

Fig. 2 Main factors influencing denitrification in cultures of potted ornamental plants



Temporal variability as observed between measurements that took place on different dates could mainly be related to climate and plant factors (Chapter 3, 4, 5). Vapour pressure deficit determined the intensity of evapotranspiration, the speed of decrease of substrate water content, and thus the duration of denitrifying activity. Temperature affected microbial activity and oxygen consumption by microbia as well as by roots.

Measurement of redox potential indicated that spatial variability, i.e. variability of N emissions among replications of one and the same measurement, mainly resulted from inhomogeneity of denitrifying conditions within potted substrate (Chapter 7). Although the denitrifying substrate volume was small (ca. 90 mL per 340 mL pot),

high variability of redox conditions was observed. It was assumed that denitrification only took place when oxygen deficiency coincided with carbon availability granted by plant roots, and that both of them occurred only in locally very limited spots.
Additionally, spatial variability resulted from variability of substrate moisture among pots before irrigation (Chapter 4). Drier substrate showed lower water content after irrigation than comparably wetter substrate. Consequently, the length of denitrifying activity was reduced.

9.2.2 Restriction of denitrification by horticultural practice

Many factors have shown impact on denitrification in the presented study (Fig. 2). While some of these are difficult or impossible to control from a practical point of view, like weather, rhizodeposition, or microbial activity, others do allow horticulturists to take influence on denitrification. Restriction of denitrification N emissions in a horticultural growing system as applied in this study may be obtained by the following strategies:

1. Increase of substrate air content (Chapter 9.2.1.1)

→ choice of substrate: high air capacity + high stability during cultivation
→ choice of pot: low diameter at pot bottom (conic, v-shaped)
→ potting practice: avoidance of substrate compaction
→ irrigation practice: short flooding periods, avoidance of waterlogging
→ climate control: low air humidity esp. after irrigation to allow evapotranspiration

2. Limitation of carbon availability in the substrate (Chapter 9.2.1.2)

→ composition of substrate: stable materials, high resistance to decomposition
→ irrigation practice: avoidance of waterlogging to reduce root exudation

3. Reasonable use of NO_3 fertilizer to reduce emission of N_2O (Chapter 9.2.1.3)

4. Restriction of denitrifying zones in areas of plant production (Chapter 9.2)

→ cleanliness: removal of substrate and plant residues from irrigated areas
→ irrigation system: regular attendance, prevention of leakage or waterlogging

10. Summary

Much research has been carried out on denitrification with regard to agricultural soils. In contrast, there is only little information available on denitrification from horticultural production of potted ornamental plants. Yet, conditions offered by greenhouse production seem favorable for denitrification because of frequent irrigation, steady NO_3 supply by fertilization, high temperatures and organic substrates for plant production. Thus, it was the aim of this study to investigate the scale of denitrification N loss and its influencing factors from cultures of potted ornamental plants. Experiments were carried out in a dynamic assay system (flow-through chambers) with planted substrate and in a closed system with unplanted substrate taking advantage of the acetylene (C_2H_2) inhibition method. The following results were obtained:

1. Acetylene (C_2H_2) application at 5 vol.% and substrate pretreatment for two hours before the experiment was found appropriate for immediate and complete inhibition of N_2O reduction in this assay system. Under these conditions, plants of *P. zonale* and *E. pulcherrima* showed side-effects of C_2H_2 after 48 h and 72 h, respectively. Yet, factors influencing denitrification, like evapotranspiration and carbon availability in the substrate, were unchanged. Because of this, and because denitrification in planted substrate usually ended within 34 hours after irrigation, side-effects of C_2H_2 application were considered not to compromise denitrification measurement.

2. Studies of unplanted substrate indicated that denitrification and emission of N_2O occurred at a noteworthy level only during oxygen deficiency. Oxygen deficiency could be induced by irrigation. Increasing N emissions at increasing glucose-C supply indicated that denitrification in peat substrate was limited by carbon availability. Nitrate supply only increased (N_2+N_2O)-N loss relative to unfertilized substrate. In contrast, the share of N_2O emissions increased with rising NO_3 supply. N emissions increased with temperature.

3. In planted substrate, substrate air and water content proved to be the most dominant factors controlling denitrification. After irrigation, at high water content, N emissions rose. The decline and end of emissions were due to the decrease of substrate water content below a threshold value. Plant and climate factors effected strong influence on substrate water content through evapotranspiration. Also, the moisture of substrate before irrigation affected water content after irrigation and thus, contributed to variability of N loss between replications.

4. In planted substrate denitrification proved to be limited by C availability. With increasing plant age of *P. zonale* (4 and 8 weeks) the course of N emissions after irrigation changed. While the duration of denitrifying activity was shortened, the height of N emission rates increased with plant age. This was presumably due to faster decrease of substrate water content and to increased root respiration and C supply by older plants, respectively. Differences in summed up N loss depending on plant age were non-significant. The contribution of increasing plant age to variability of N emissions during cultivation was considered to be low.

5. Substrate properties affected denitrification through air and water capacity. N loss and water content after irrigation were decreased by dry relative to moist substrate before irrigation. Substrate compaction increased water content after irrigation and N loss relative to uncompacted substrate. Sieving of substrate increased N emissions although no difference in water content was observed. Thus, the increase in N loss was presumably due to changes in pore structure or increased shrinkage of the sieved substrate. Addition of wood fibres or rice husks to sieved peat (30 vol.% : 70 vol.%) did not significantly change N loss although both unplanted mixtures showed higher air capacity than pure peat. It was assumed that air capacity of the mixtures deteriorated because of decomposition and shrinkage during plant cultivation.

6. Within potted substrate, the layer close to the pot bottom showed highest water content after irrigation and thus, offered best conditions for denitrification. This was confirmed by measurement of redox potential. Low

redox potential allowing denitrification was found only in the lower substrate layer and up to 2.5 cm from the pot bottom. Yet, not all electrodes placed close to the pot bottom showed decreasing potential after irrigation. It was assumed that supply of carbon by plant roots was necessary in addition to high water content to cause denitrifying activity and decrease of redox potential. Measurement of redox potential indicated that high variability of conditions for denitrification existed within one pot. This contributed to explaining the high variability of N loss.

7. Horticultural practice / management influenced denitrification N loss to some extent. N emissions decreased when irrigation time was reduced from two hours to 30 minutes. The use of pots having a lower diameter at the pot bottom than at the top (conic, v-shaped) reduced N emissions relative to more cylindric or u-shaped pots. This was presumably due to the smaller volume of the lower substrate layer in the v-shaped pot. The same reason was assumed for higher N loss from 550 mL pots relative to 240 mL pots. But there, differences in N loss vanished when irrigation frequency was considered in addition to N loss per irrigation. Postponing irrigation from morning to afternoon changed the course of N emissions, but did not change total N loss. The amount of bottom holes only slightly affected N loss. In contrast, notable N emissions occurred from irrigation mat when used withouth protective plastic film.

8. Extrapolated denitrification N loss (N_2+N_2O) from cultivation of potted plants in an ebb/flood irrigation system as presented in this study amounted to 6.9 kg ha^{-1} $year^{-1}$. N_2O-N emissions were estimated to be 2.4 kg ha^{-1} $year^{-1}$. Thus, monetary loss of N-fertilizer as well as the contribution of pot plant production to emission of environmentally harmful N_2O from was considered to be low.

11. Zusammenfassung

Viele Untersuchungen haben sich mit der Denitrifikation in landwirtschaftlichen Böden beschäftigt. Im Gegensatz dazu gibt es nur wenige Informationen über Denitrifikation in der gartenbaulichen Topfpflanzenproduktion. Die Bedingungen für die Denitrifikation im Unterglasanbau erscheinen jedoch günstig aufgrund regelmäßiger Bewässerung, kontinuierlicher NO_3-Versorgung durch Fertigation, hoher Temperatur und organischer Substrate für die Pflanzenanzucht. Daher war es das Ziel dieser Arbeit, Ausmaß und bestimmende Faktoren der N-Verluste durch Denitrifikation in getopften Zierpflanzenkulturen zu untersuchen. Mithilfe der Acetylen-(C_2H_2)-Inhibierungsmethode wurden Experimente in dynamischen Versuchsanlagen (Durchflußkammern) mit bepflanztem Substrat, sowie in geschlossenen Versuchsgefäßen mit unbepflanztem Substrat durchgeführt. Folgende Ergebnisse wurden erzielt:

1. Eine Acetylene-Konzentration von 5 vol.% und eine Vorbehandlungsdauer des Substrats von zwei Stunden erwiesen sich im gewählten Versuchssystem als geeignet für die sofortige und vollständige Hemmung der N_2O-Reduktion. Hierbei zeigte C_2H_2 nach 48 bzw. 72 Stunden Nebenwirkungen auf die Versuchspflanzen *P. zonale* und *E. pulcherrima*. Dagegen wurden keine Auswirkungen auf Einflußfaktoren der Denitrifikation beobachtet, wie z.B. Evapotranspiration oder Kohlenstoff-Verfügbarkeit im Substrat. Deshalb, und weil die Denitrifikation in bepflanztem Substrat üblicherweise innerhalb von 34 h endete, wurde von keiner Beeinträchtigung der Denitrifikationsmessungen durch Nebenwirkungen von C_2H_2 ausgegangen.

2. Untersuchungen von unbepflanztem Substrat zeigten, daß Denitrifikation und Emission von N_2O nur bei Sauerstoffmangel in nennenswertem Umfang stattfanden. Sauerstoffmangel wurde durch Anstaubewässerung induziert. Steigende N Emissionen mit steigendem Glukose-C-Angebot deuteten auf eine C-Limitierung der Denitrifikation in Torfsubstrat. Das Nitrat-Angebot erhöhte (N_2+N_2O)-N-Verluste nur gegenüber der ungedüngten Kontrolle. Im Gegensatz dazu nahm der N_2O-Anteil der N-Emissionen mit steigendem NO_3 Angebot zu. N-Emissionen stiegen mit der Temperatur.

3. In bepflanztem Substrat erwiesen sich Substratluft- bzw. -wassergehalt als stärkste Einflußfaktoren der Denitrifikation. Nach Bewässerung, bei hohem Wassergehalt, stiegen die N Emissionen an. Ihr Sinken und Enden wurde auf das Absinken des Substratwassergehalts unter einen Schwellenwert zurückgeführt. Pflanzen- und Klimafaktoren übten über die Evapotranspiration einen starken Einfluß auf den Substratwassergehalt aus. Auch die Substratfeuchte vor Bewässerung beeinflußte den Wassergehalt nach Bewässerung und trug damit zur Variabilität der N-Verluste innerhalb einer Messung bei.

4. Die Denitrifikation war auch in bepflanztem Substrat C-limitiert. Mit steigendem Pflanzenalter von *P. zonale* (4 bzw. 8 Wochen) veränderte sich der Verlauf der N-Emissionen nach Anstau. Bei verkürzter Denitrifikationsdauer stieg die Höhe der N-Emissionen an. Dies lag vermutlich am schnelleren Absinken des Substratwassergehalts bzw. an der höheren Wurzelatmung und C-Abgabe durch ältere Pflanzen. Unterschiede im summierten N-Verlust in Abhängigkeit des Pflanzenalters waren nicht signifikant. Der Beitrag des Pflanzenalters zur Variabilität der N-Emissionen im Kulturverlauf wurde als gering eingeschätzt.

5. Substrateigenschaften beeinflußten die Denitrifikation durch ihre Wirkung auf die Wasser- und Luftkapazität. N-Verluste und Wasserkapazität waren bei vor Bewässerung trockenem Substrat geringer als bei vor Bewässerung feuchtem Substrat. Substratverdichtung erhöhte sowohl Wassergehalt nach Anstau als auch N-Verluste im Vergleich zu unverdichteten Substrat. Das Sieben von Substrat steigerte die N-Emissionen, obwohl keine Veränderung im Wassergehalt beobachtet wurde. Vermutlich war der Anstieg der N-Verluste auf Veränderungen in der Porenstruktur oder auf erhöhtes Schrumpfen des gesiebten Substrats zurückzuführen. Die Beimischung von Holzfasern oder Reisspelzen zu gesiebtem Torf (30 vol.%:70 vol.%) bewirkte keine signifikante Reduktion der N-Verluste, obwohl die unbepflanzten Substratmischungen eine höhere Luftkapazität als reiner Torf aufwiesen. Es wurde angenommen, daß die Luftkapazität der Substratmischungen aufgrund von Zersetzung und Schrumpfung während der Kulturdauer abnahm.

6. Innerhalb des getopften Substrats wies die unterste Schicht am Topfboden den höchsten Wassergehalt nach Anstaubewässerung auf und bot damit die besten Bedingungen für die Denitrifikation. Dies wurde durch Messungen des

Redoxpotentials bestätigt. Niedrige Redoxpotentiale wurden nur in der unteren Substratschicht bis 2,5 cm über dem Topfboden gemessen. Dennoch zeigten nicht alle nah am Topfboden positionierten Elektroden ein Absinken des Potentials nach Anstau. Vermutlich war zusätzlich zum hohen Wassergehalt die Verfügbarkeit von Kohlenstoff aus Pflanzenwurzeln notwendig, um ein Absinken des Redoxpotentials und Denitrifikation zu ermöglichen. Messungen des Redoxpotentials wiesen auf eine hohe Variabilität der Denitrifikationsbedingungen innerhalb eines Topfes und trugen damit zur Erklärung der hohen Variabilität von N-Emissionen bei.

7. Maßnahmen der gärtnerischen Kulturführung beeinflußten die Denitrifikation zum Teil. N-Emissionen sanken durch Kürzung der Bewässerungszeit von zwei Stunden auf 30 Minuten. Die Verwendung von Töpfen mit geringeren Durchmesser am Boden als am oberen Rand (konisch, v-förmig) im Vergleich zu zylindrischen oder u-förmigen Töpfen mit gleichem Fassungsvermögen reduzierte die N-Verluste. Dies lag vermutlich an dem geringeren Volumen der untersten Substratschicht in den v-förmigen Töpfen. Dieselbe Ursache wurde für höhere N-Verluste aus 550 mL- im Vegleich zu 240 mL-Töpfen vermutet. Hierbei unterschieden sich die Topfgrößen jedoch nicht, wenn zusätzlich zum N-Verlust pro Bewässerung die Bewässerungshäufigkeit berücksichtigt wurde. Die Verlegung der Bewässerung vom Morgen in den Nachmittag veränderte den Verlauf der N-Emissionen, aber nicht den gesamten N-Verlust. Die Anzahl der Topfbodenlöcher beeinflußte die N-Emissionen nur geringfügig. Dagegen wurden nennenswerte N-Emissionen aus Bewässerungsmatten, die ohne schützende Nadelfolie benutzt wurden, gemessen.

8. Hochgerechnete N-Verluste durch Denitrifikation aus der Kultivierung von Topfpflanzen in einem Ebbe/Flut-System betrugen als (N_2+N_2O)-N 6,9 kg ha^{-1} $Jahr^{-1}$ und als N_2O-N 2,4 kg ha^{-1} $Jahr^{-1}$. Sowohl der monetäre Verlust von Düngerstickstoff als auch der Beitrag der Topfpflanzenproduktion zur Emission des umweltschädlichen N_2O wurden als gering bewertet.

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