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Typha latifolia paludiculture effectively improves water quality and reduces greenhouse gas emissions in rewetted peatlands

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ABSTRACT

Paludiculture, the cultivation of crops on wet or rewetted agricultural peatlands, sustainably integrates productive land use with the provision of multiple ecosystem services. Paludiculture crops thrive under waterlogged conditions that stimulate nitrogen (N) and phosphorus (P) removal from soil and water and convert serious drainage-induced carbon (C) losses to C sequestration. Nutrient uptake by paludicrops can prevent mobilisation after rewetting and provide opportunities for purification of nutrient-rich water. Uncertainty remains, however, if and to what extent N loading and a subsequent increase in biomass productivity affect nutrient cycling as well as emissions of the potent greenhouse gases methane (CH₄) and nitrous oxide (N₂O). In this study, we use mesocosms with rewetted peat to investigate the effect of different N sources in surface water on biomass production of *Typha latifolia*, a typical paludiculture crop, and the emissions of CH₄ and N₂O. Organic (*Azolla filiculoides*; urea) or mineral (KNO₃; NH₄NO₃) N was supplied either a single time (steady state) or repeatedly (pulse) to simulate a total surface water load of 150 kg N ha⁻¹. We found that N stimulated aboveground and belowground biomass production and nutrient uptake by *T. latifolia*. These effects were absent in *Azolla* treatments. Whereas after two months CH₄ emissions arose to substantial amounts (> 10 mg CH₄ m⁻² day⁻¹) in unvegetated mesocosms loaded with organic N, they remained very low (< 1 mg CH₄ m⁻² day⁻¹) in vegetated mesocosms, despite the labile C pool in the extensive belowground biomass and organic N loading. Overall, N₂O emissions were close to zero and were only detected episodically after NO₃⁻ loading, irrespective of plant presence. Our findings support that *T. latifolia* as a paludicrop effectively removes various forms of N and P when harvested, and strongly mitigates CH₄ emission after the rewetting of agricultural peat soils compared to unvegetated conditions.

1. Introduction

Peatlands worldwide are vast carbon (C) storages containing > 30% of the earth's soil C (Lamers et al., 2015). The majority of undisturbed peatlands act as net carbon dioxide (CO₂) sinks and methane (CH₄) sources (Frolking et al., 2011). Drainage of peat soils, for instance for agricultural purposes, results in reduced CH₄ emissions but increased CO₂ and N₂O emissions, usually leading to net greenhouse gas (GHG) emissions (Haddaway et al., 2014; Kasimir-Klemetsson et al., 1997; Martikainen et al., 1993). Currently, peatlands that are drained for agricultural purposes contribute to an estimated 6% of the global

anthropogenic carbon dioxide (CO₂) emission, while only covering 0.3% of the earth's land surface (Joosten et al., 2012). Rewetting of drained peatlands is a much promoted mitigation measure to reduce GHG emissions (Wilson et al., 2016). Rewetting reduces CO₂ and N₂O emissions through restoring the peatland's function as a C sink, but enhances CH₄ emissions as a result of anoxia (Karki et al., 2016; Tanneberger and Wichtmann, 2011). As a consequence, the success of rewetting as a climate change mitigation measure depends on the balance between decreased CO₂ and N₂O emissions versus enhanced CH₄ emissions (Van de Riet and Hefting, 2013). Besides CH₄ emissions, rewetting can lead to nutrient mobilization with subsequent

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eutrophication and can result in the loss of productive land (Harpenslager et al., 2015; Zak and Gelbrecht, 2007). To restore multiple ecosystem services of peatlands while maintaining productive land use, the cultivation of perennial crops on wet or rewetted peatlands (paludiculture) is a promising, much more sustainable alternative to drainage (Wichtmann et al., 2016). Biomass production of so-called “paludicrops”, such as *Typha* sp., may well exceed the productivity of degraded, nutrient-rich peatlands and surface waters in agricultural areas (Wichtmann et al., 2016; Wild et al., 2001). However, little is known about the productivity of paludiculture and subsequent effects on greenhouse gas emissions and nutrient cycling in rewetted peatlands.

The presence of vegetation, including crops used in paludiculture, can positively or negatively affect CH_4 emissions (Bhullar et al., 2014; Christensen et al., 2003; Couwenberg et al., 2011; Henneberg et al., 2016). Aerenchymous tissue in wetland plants can provide a transport pathway connecting anoxic soil layers to the atmosphere, resulting in radial oxygen loss (ROL) in the rhizosphere (Colmer, 2003; Couwenberg and Fritz, 2012; Wiefśner et al., 2002). In addition to this diffusion pathway, O_2 can be transported by the plant through convective flow-through as a result of a pressure difference created by photosynthesis (Stottmeister et al., 2003). The subsequent availability of O_2 in the rhizosphere promotes CH_4 oxidation by methanotrophs and simultaneously inhibits methanogenesis (Frenzel et al., 1999; Fritz et al., 2011). On the other hand, the aerenchyma can act as a direct diffusion pathway for CH_4 from anoxic soil layers to the atmosphere (Bhullar et al., 2013; Chanton et al., 1993; Schimel, 1995). Furthermore, plants can fuel methanogenesis by the provision of carbon substrate through root exudates (e.g. organic acids) as well as decaying organic matter (Ström et al., 2005). The effects of different plant species on CH_4 emissions in wetlands are complex and challenging to predict (Carmichael et al., 2014). Accordingly, it is crucial to assess the effect of paludicrops on CH_4 emissions in order to select optimal species for paludiculture.

Besides being a C sink, peatlands can store large amounts of nitrogen (N) and phosphorous (P) due to the slow turnover of organic matter under anoxic conditions (Reddy and DeLaune, 2008). Rewetting of degraded peatlands often results in the mobilization of these nutrients, mostly as ammonium (NH_4^+) and phosphate (PO_4^{3-}), which accumulate in pore water and surface water and can cause eutrophication (Harpenslager et al., 2015; Smolders et al., 2006; Van de Riet and Hefting, 2013; Zak and Gelbrecht, 2007). Rapid uptake of these nutrients by paludiculture crops may well prevent this mobilization and also provide the option to remove nutrients by harvesting biomass (Giannini et al., 2017; Ryciewicz-Borecki et al., 2017; Schulz et al., 2011). As inundated peatlands are often N limited due to high N losses to the atmosphere (Reddy and DeLaune, 2008; Van Duren and Peggel, 2000), simultaneous biomass production and nutrient uptake from soil and water can be promoted by the addition of N rich surface water (e.g. agricultural runoff) (Cabezas et al., 2012; Grace, 1988). In this sense, paludiculture can provide a water purification function similar to a free water surface (FWS) constructed wetland with emergent plants (Vymazal, 2007), while sustainable paludicrop production can be realized without depleting all available N-pools. N removal can occur via gaseous losses (denitrification, volatilisation) or incorporation in plant biomass followed by a harvest. Conversion of N can occur through processes such as ammonification and nitrification, and will depend on the availability of electron acceptors. Ultimately, the fate of N in paludiculture will depend on its initial form (i.e. NO_3^- , NH_4^+ or organic N), water and soil characteristics (e.g. pH, substrate quality, temperature and redox potential) and the presence of biota (plants, microbes). The extent and pathways of N removal in paludiculture need to be further investigated in order to define its potential for water purification.

Next to affecting nutrient cycling and productivity, surface water nutrient composition can affect GHG emissions. N may either inhibit or

stimulate CH_4 oxidation as well as production, depending on N form and concentration, soil type and availability of other nutrients (Bodelier and Laanbroek, 2004; Singh and Strong, 2016; Veraart et al., 2015; Zhu et al., 2012). Also, NO_3^- loading can induce N_2O emissions as a result of incomplete denitrification (Maltais-Landry et al., 2009). In the end, C and N cycling in paludiculture will depend on the chemical composition of inflow water, including the forms of nitrogen and decomposable C (Mander et al., 2014a; Singh and Strong, 2016). It is therefore vital to investigate what role paludicrops play in CH_4 and N_2O emissions of a wet peatland, and how CH_4 and N_2O emissions can be minimized by controlling water quality.

In the present study, we aim to investigate the effects of surface water N composition (NO_3^- , NH_4^+ , urea and N from decaying biomass) on plant growth, N and P cycling, and emissions of CH_4 and N_2O in a paludiculture system. To achieve this under controlled conditions, we simulated peat rewetting in mesocosms that were either unvegetated or planted with *Typha latifolia* – a potential paludiculture crop (Wichtmann et al., 2016). N loads were supplied in either a steady state or pulsing flow regime to imitate constant supply or periodical renewal of N rich water, respectively, in order to investigate the effect of possible management practices on biomass yield, nutrient cycling and GHG emissions. Results will be discussed with respect to paludiculture with *T. latifolia*, the nutrient loads required for sustainable production and its potential to provide ecosystem services (C sequestration, water purification, CH_4 and N_2O emission reduction) compared to unvegetated rewetted peat.

2. Materials and methods

2.1. Peat characteristics

Peat soil was collected in Gersloot, Friesland (53.02°N, 5.93°E), a peat excavation site in the Netherlands. As the fen peat soil was collected from a depth of several metres, it was nutrient-poor, thus preventing a large background effect of peat soil in our experiment. Lime (5 g Dolokal L⁻¹ soil; SIBELCO Ankerpoort NV, Maastricht, The Netherlands) was added to the soil in order to increase soil pH from 3.6 to 5.7 to avoid negative effects on plant growth (Van Diggelen et al., 2015). The peat was homogenized using a concrete mixer and a bulk sample was taken to characterize the chemical composition of the soil prior to the experiment. Then, 72 mesocosms (opaque PVC pipes, diameter 15 cm, height 30 cm) containing a plastic bag to contain the soil and water were filled with peat up to 25 cm.

2.2. Vegetation

T. latifolia was grown at the Radboud greenhouse facilities from seeds collected in a ditch near Cuijk, The Netherlands. In 45 mesocosms, three 8–10 cm tall seedlings of *T. latifolia* were planted. During the first two weeks, non-viable plants were replaced by healthy individuals. The remaining mesocosms contained only peat soil (unvegetated, n = 27). Unwanted germinating plants were removed by hand.

2.3. Abiotic conditions

All mesocosms were placed in a temperature-regulated water bath in the Radboud greenhouse facilities, kept at 15 °C by a cryostat (ThermoFlex 1400, Thermo Fisher Scientific, Waltham, MA, USA). Rewetting of soils was simulated by adding demineralized water to a level of 5 cm above the soil surface, following growth of the plants to prevent submersion. Light conditions (irradiance) were maintained at 186 W m⁻² or higher for 16 h per day, using grow lights if sunlight was not sufficient. Average daytime air temperature in the greenhouse was 22.3 °C in March, 22.7 °C in April, and 24.9 °C in May. The average relative humidity (RH) in daytime was 40%. Demineralized water was

added to the rim of the PVC tubes every other day to maintain a stable water level of 5 cm above soil surface. Mesocosms were acclimatized under these conditions for eight days prior to the nitrogen treatments. During the experiment, mesocosm soil temperature at 5 cm depth varied between 13.9 °C and 16.9 °C with an average of 14.9 °C. The pH of surface water and pore water was 6.75 ± 0.56 and 6.24 ± 0.16 (average $\pm$ sd), respectively. Soil C:N ratio was 45.8, with extractable NO_3^- of $1010 \mu\text{mol L}^{-1}$, NH_4^+ of $672 \mu\text{mol L}^{-1}$, P of $7.84 \mu\text{mol L}^{-1}$ and K of $395 \mu\text{mol L}^{-1}$ (all concentrations per litre fresh soil). The experiment ran from the 22th of February 2017 till the 1st of June 2017 (99 days).

2.4. Nitrogen treatments

Mesocosms were divided into nine groups, including eight different N treatment groups and one control group (no N added). All N treatments received 150 kg N ha^{-1} ($0.27 \text{ g N per mesocosm}$), based on realistic concentrations in farm runoff (Geurts et al., 2017). Four different N sources were used, including two mineral forms and two organic forms: potassium nitrate (KNO_3), ammonium nitrate (NH_4NO_3), urea ($\text{CO}(\text{NH}_2)_2$) and *Azolla filiculoides* (either as living biomass or as dried litter; from here on referred to as *Azolla*). *Azolla*, an aquatic fern, fixates dinitrogen (N_2) and is therefore commonly used as a biofertilizer in rice paddies (Singh and Strong, 2016). We added living *Azolla* grown in the greenhouse in a single layer which covered the water surface. *Azolla* litter was created from the same stock by drying for four days at 70 °C. C:N ratios of *Azolla* litter were determined prior to the experiment (methods below) and added weights were calculated accordingly. The remaining treatments were applied either as slow-release granules which released nutrients over a time span of 3 months (slow-release) as KNO_3 , urea (Haifa Group, Haifa, Israel) or NPK (The Scotts Company, Marysville, OH, USA) or in solution (pulse) as KNO_3 , NH_4NO_3 (Duchefa Biochemie, Haarlem, The Netherlands) or urea (Merck, Kenilworth, NJ, USA) resulting in eight different N treatments. Slow-release granules were applied once at the start of the experiment, whereas pulses were added thrice (day 14, 38 and 62) during the experiment, to mimic a stable state or pulsing regime, respectively. All treatments with exception of slow release NH_4NO_3 (given as NPK), received a weekly dose of K_2HPO_4 (Merck, Kenilworth, NJ, USA) in solution to avoid limitation of P and K, resulting in a total P load of 37 kg ha^{-1} (N:P of 4) and a total K load of 94 kg ha^{-1} . To correct for the extra K in the KNO_3 treatment, all other treatments received an additional weekly dose of KCl (Duchefa Biochemie, Haarlem, The Netherlands) in solution resulting in a total K load of 190 kg ha^{-1} (N:K of 0.8) in all treatments.

2.5. CH_4 and N_2O emissions

CH_4 and N_2O fluxes were measured eleven times throughout the experiment in weekly or biweekly intervals: two zero measurements before N application (day 2 and 7), twice after the first application (pulse and slow-release) (day 21 and 35), thrice after the second pulse (day 46, 53 and 60) and four times after the third pulse (day 68, 75, 82 and 96). Measurements were carried out between 8 AM and 4 PM, and the order was randomized to account for diurnal variability. Soil temperature at 5 cm depth was recorded for each measurement. Net ecosystem exchange of CH_4 and N_2O was measured using static, airtight chambers. A dark PVC chamber (15 cm inner diameter), equipped with a temperature logger (HOBO, Onset Computer Corporation, Bourne, MA, USA) and a battery-driven fan was sealed to each mesocosm consecutively. This chamber was linked to a Picarro G2508 Greenhouse Gas Analyzer (Picarro Inc., Santa Clara, CA, USA) through fine plastic tubing (0.4 cm inner diameter) via two gas-tight ports in a closed loop. Measurements usually lasted 180 s and included only diffusive fluxes: measurements were repeated if ebullition was observed. CH_4 and N_2O fluxes ($\text{mg m}^{-2} \text{ d}^{-1}$) were calculated according to Almeida et al. (2016) using the following equation:

$$F = \frac{V}{A} * \text{slope} * \frac{P * M * F1}{R * T}$$

Where F is gas flux ($\text{mg m}^{-2} \text{ d}^{-1}$), V is chamber volume (m^3), A is chamber surface area (m^2), slope is the slope of the measured CH_4 or N_2O concentration in time (ppm s^{-1}) as calculated in Microsoft Excel (2016); P is the atmospheric pressure (kPa); M is the molecular mass of CH_4 or N_2O (g mole^{-1}); F1 is the conversion factor of seconds to days; R is the gas constant ($8.3144 \text{ J K}^{-1} \text{ mol}^{-1}$); and T is temperature (K).

2.6. Water sampling and analysis

Pore water was extracted five times throughout the experiment using Rhizon samplers (Rhizosphere Research Products, Wageningen, The Netherlands) attached to a syringe under vacuum. Surface water was collected in vials nine times during the experiment. After pH and alkalinity were determined with a Ag/AgCl electrode (Orion Research, Beverly, MA, USA) and TIM 840 Titration Manager (Radiometer Analytical SAS, Villeurbanne, France), water samples were stored at 4 °C (adding 0.1 mL of 65% HNO_3 to a 10 mL subsample) and -20 °C until further analysis. CH_4 concentrations in the pore water were measured one day prior to harvest using gas chromatography. Additional pore water samples were collected in gastight glass bottles with a septum at atmospheric pressure and room temperature (21 °C). CH_4 was measured in the headspace of these bottles using an HP 5890 gas chromatograph equipped with a Porapak Q column (80/100 mesh) and a flame ionization detector (GC-FID, Hewlett Packard, Palo Alto, CA, USA), and original concentrations in the pore water were calculated using Henry's law (Sander, 2015).

2.7. Soil sampling and analysis

Soil samples were taken prior to the experiment as well as after plants were harvested (day 99). Samples were collected along the total depth (25 cm) of the mesocosm and homogenized. To determine bio-available NH_4^+ and NO_3^- , salt extractions were carried out using 17.5 g of fresh soil, incubated with 50 mL of 0.2 M sodium chloride (NaCl). After 120 min of incubation on a shaker at 105 rpm, pH was determined and fluid was extracted using Rhizon samplers and bottles under vacuum. After extraction, fluid samples were stored at -20 °C until further analysis. In addition, subsamples of fresh soil were dried at 70 °C for 48 h to determine dry weight and bulk density. Dry soils were ground and homogenized using a ball-mill (Fritsch Pulverisette Ball Mill, Fritsch GmbH, Idar-Oberstein, Germany) at 400 RPM for 4 min. Bio-available P (Olsen-P) was determined by incubating 3g dried soil in 60 mL 0.5 M sodium carbonate (NaHCO_3) for 30 min at 105 RPM, followed by extractions as above (Henriksen, 1965). Total P (TP), Total K (TK) and other nutrients were determined by digestion, and total nitrogen (TN) was measured using a CNS analyser (methods described below).

2.8. Biomass growth and analysis

Leaf number and average length of *T. latifolia* were recorded weekly. All aboveground plant biomass was harvested after 99 days, dried at 70 °C for four days and ground. Belowground biomass was harvested as well, rinsed with water to remove soil, divided into roots and rhizomes, then dried and ground similarly. TP, TK and TN were determined by the methods described below. An N budget was calculated by subtracting final amounts in different compartments (soil, surface water, below- and aboveground biomass) from N added and initial available N (NO_3^- and NH_4^+ from salt extractions) in the soil.

2.9. Chemical analysis

Concentrations of ammonium (NH_4^+), nitrate (NO_3^-), phosphate

(PO_4^{3-}) and chloride (Cl^-) were determined by colorimetric methods (Auto Analyser III, Bran and Luebbe GmbH, Norderstedt, Germany) in the samples that were stored at -20°C (Geurts et al., 2008). Potassium (K) and sodium (Na) concentrations were analysed using flame photometry (FLM3 Flame Photometer, Radiometer, Copenhagen, Denmark). Inductively coupled plasma emission spectrometry (ICP-OES) was used to measure major elements (Ca, K, Mg, Na, P, S) and trace elements (Al, Fe, Mn, Si, Zn) (IRIS Intrepid II, Thermo Electron corporation, Franklin, MA, USA) in the samples that were stored at 4°C . TN was determined in dry soil and plant material (3 mg) using an elemental CNS analyser (NA 1500, Carlo Erba; Thermo Fisher Scientific, Franklin, USA). TP and TK were determined after microwave digestion, adding 4 mL HNO_3 (65%) and 1 mL H_2O_2 (35%) to 200 mg dried soil in Teflon vessels, followed by heating in an EthosD microwave (Milestone, Sorisole Lombardy, Italy).

2.10. Data analysis

Statistical analysis was performed using R v. 3.3.2 (R Core Team, 2017). Differences in biomass yield, plant height and N content between treatments were tested by means of one-way ANOVA and subsequent Tukey post-hoc test. Pore water and surface water nutrient concentrations were compared using a general linear model with treatment, plant presence, time and their interactions as fixed effects. CH_4 data were split in two time series: before and after the onset of CH_4 emissions (day 60). These series were analysed separately. CH_4 fluxes were log-transformed after the addition of a constant (the lowest measured flux) to approach a normal distribution of residuals and improve variance homogeneity. N source, vegetation (presence/absence of *T. latifolia*), time and their interactions were included as fixed effects in a linear model. Temporal autocorrelation was tested for but did not improve AIC values. We used a significance level of $\alpha = 0.05$ in all cases.

3. Results

3.1. Plant growth, biomass yield and nutrient uptake

At the start, plant length ranged between 11.3 and 17.0 cm, with an average of 14.3 cm. In the course of the experiment, *T. latifolia* plants grew in all treatments. Plants receiving inorganic N or urea attained a significantly greater average length, belowground and aboveground biomass than the unfertilized control and *Azolla* treatments ($P < 0.001$ in both cases) (Fig. 1, Fig. 2). The addition of KNO_3 in pulses resulted in a lower total biomass production ($24.4 \text{ g DW mesocosm}^{-1}$) than the

Fig. 1. Average length of *T. latifolia* in different N treatments during the experiment. Treatments receiving mineral N resulted in enhanced length of *T. latifolia* compared to control and *Azolla* treatments ($P < 0.001$). Error bars depict standard deviations.

Fig. 2. Average biomass yield (aboveground and belowground) of *T. latifolia* in different treatments. SR stands for slow-release, P stands for pulse. Different letters indicate significant differences between treatments ($P < 0.05$). Error bars represent standard deviations.

urea treatment ($30.2 \text{ g DW mesocosm}^{-1}$) ($P < 0.05$). Extrapolated aboveground biomass yields after 100 days ranged from 1.9 to 2.6 tons ha^{-1} for controls (no N supply) and from 4.7 to 10.3 tons ha^{-1} (2–5 times increase) after addition of mineral N. Plants in control treatments had a lower N content than those in N treatments (0.55% versus 0.69%, $P < 0.001$), but N treatments did not differ. N standing stock was therefore proportional to biomass for plants receiving N, with urea and NH_4NO_3 in pulses resulting in the highest N standing stocks (on average $102 \text{ mg mesocosm}^{-1}$) (Fig. 3). In both vegetated and un-vegetated mesocosms, final N quantities are lower than initial N quantities (Table 1). N loss was 88% (slow-release NH_4NO_3) to 99% (slow-release KNO_3) in un-vegetated mesocosms. In vegetated mesocosms receiving N (excluding *Azolla* in which added N amounts were uncertain), plants took up 41% (KNO_3 pulse) to 53% (urea pulse) of the initially available and added N, and the remaining N load was largely lost as well (59% to 47% N loss, accordingly). Extrapolated N removal by plants ranged between 10 kg ha^{-1} (control) to 75 kg ha^{-1} (urea pulse). Average N:P ratios ranged from 3.4 (control and *Azolla* litter) to 6.0 (slow release NH_4NO_3). P standing stock (Fig. 4) was higher in all mineral N treatments compared to the control, with average extrapolated P removal ranging from 2.7 kg ha^{-1} (control) to 15 kg ha^{-1} (urea in pulses).

3.2. Pore water

NH_4^+ concentrations in pore water showed contrasting trends depending on plant presence and N treatment (Fig. 5). During the

Fig. 3. N standing stock of *T. latifolia* per treatment. SR: slow-release, P: pulse. Different letters indicate significant differences between treatments ($P < 0.05$). Error bars represent standard deviations.

Table 1

Nitrogen budget for treatments with and without *T. latifolia*. SR: slow-release, P: pulse. Values are given as mean $\pm$ standard deviation, in mg N mesocosm⁻¹. Net N loss is calculated as start values subtracted from end values in soil (plant available N determined by salt extraction), water and plant fractions. Percentages indicate the proportion of N available at the start, that is lost from the system, i.e. not found in any fraction in the end (e.g. gaseous losses). In living *Azolla* treatments, an N load could not be estimated and the N load delivered by *Azolla* litter decomposition is probably overestimated, which complicates budget calculation in *Azolla* treatments.

		Start (t = 0)		End (t = 99)						
		Load	Soil	Soil	Surface water	Aboveground biomass	Belowground biomass	Net N loss	% N loss	
Unvegetated	Control	0	104	9.59 $\pm$ 3.39	0.1 $\pm$ 0.12			94.31	90.68	
	<i>Azolla</i> living	N.A.	104	5.35 $\pm$ 4.42	0.42 $\pm$ 0.2			98.23	94.45	
	<i>Azolla</i> litter	265	104	16.43 $\pm$ 6.82	0.32 $\pm$ 0.02			352.25	95.46	
	KNO ₃ SR	265	104	1.71 $\pm$ 0.28	0.39 $\pm$ 0.2			366.90	99.43	
	KNO ₃ P	265	104	8.47 $\pm$ 4.58	0.22 $\pm$ 0.11			360.31	97.64	
	NH ₄ NO ₃ SR	265	104	41.81 $\pm$ 59.89	1.03 $\pm$ 1.61			326.16	88.39	
	NH ₄ NO ₃ P	265	104	5.43 $\pm$ 3.03	0.3 $\pm$ 0.22			363.27	98.45	
	Urea SR	265	104	33.34 $\pm$ 32.29	0.21 $\pm$ 0.14			335.45	90.91	
	Urea P	265	104	27.93 $\pm$ 8.82	0.2 $\pm$ 0.08			340.87	92.38	
<i>T. latifolia</i>	Control	0	104	3.63 $\pm$ 6.08	0.07 $\pm$ 0.08	21.38 $\pm$ 2.87	30.14 $\pm$ 8.82	48.61	46.74	
	<i>Azolla</i> living	N.A.	104	2.53 $\pm$ 2.19	0.51 $\pm$ 0.32	38.02 $\pm$ 4.65	31.18 $\pm$ 16.44	18.96	18.23	
	<i>Azolla</i> litter	265	104	6.68 $\pm$ 7.16	0.13 $\pm$ 0.07	31.36 $\pm$ 3.35	43.98 $\pm$ 13.91	299.65	81.21	
	KNO ₃ SR	265	104	2.63 $\pm$ 2.76	0.11 $\pm$ 0.1	86.93 $\pm$ 13.55	99.75 $\pm$ 17.08	179.58	48.67	
	KNO ₃ P	265	104	1.43 $\pm$ 1.2	0.66 $\pm$ 0.03	80.78 $\pm$ 13.43	69.98 $\pm$ 8.71	216.15	58.58	
	NH ₄ NO ₃ SR	265	104	1.28 $\pm$ 1.06	0.04 $\pm$ 0.02	81.76 $\pm$ 7.88	85.93 $\pm$ 12.79	199.99	54.20	
	NH ₄ NO ₃ P	265	104	1.18 $\pm$ 1.1	0.06 $\pm$ 0.04	91.84 $\pm$ 16.43	87.9 $\pm$ 17.17	188.02	50.95	
	Urea SR	265	104	6.84 $\pm$ 6.68	0.03 $\pm$ 0.01	74.63 $\pm$ 6.01	86.86 $\pm$ 22.29	200.64	54.37	
	Urea P	265	104	1.34 $\pm$ 1.26	0.04 $\pm$ 0.01	112.8 $\pm$ 15.94	82.54 $\pm$ 13.23	172.28	46.69

Fig. 4. P standing stock of *T. latifolia* per treatment. SR: slow-release, P: pulse. Different letters indicate significant differences between treatments ($P < 0.05$). Error bars represent standard deviations.

experiment, pore water NH₄⁺ concentrations decreased in all vegetated treatments and NH₄⁺ was depleted within 60 days ($P < 0.001$). At the end of the experiment, all vegetated treatments had lower N concentrations than treatments without plants ($P < 0.001$). In mesocosms without plants, NH₄⁺ concentrations remained stable, around 200 μ M, for KNO₃, *Azolla* and control treatments, which is comparable to concentrations previously found in slightly to moderately degraded peat after rewetting (Zak and Gelbrecht, 2007). In NH₄NO₃ and urea treatments an increase was observed after the first application ($P < 0.001$ in all cases), with concentrations up to 1240 μ M (slow-release urea), indicating NH₄⁺ accumulation in pore water. NH₄⁺ concentrations in these treatments decreased during the experiment, but were still higher (around 400 μ M on average) than all other treatments at the end of the experiment. Pore water NO₃⁻ was depleted in all treatments within 60 days ($P < 0.001$; Fig. S1). Slow-release KNO₃ treatments showed a high peak in pore water NO₃⁻ after application, and NO₃⁻ concentrations in these treatments remained slightly elevated (final concentrations on average 3.47 μ mol L⁻¹ compared to 0.26 μ mol L⁻¹ in controls) throughout the experiment in mesocosms without plants ($P < 0.001$). Pore water P concentrations remained relatively stable in unvegetated

treatments and increased up to four times in *Azolla* litter treatments, then decreased again (Fig. S2). *T. latifolia* depleted all pore water P, except in control and *Azolla* treatments. Final pore water pH was higher in vegetated than in unvegetated mesocosms (on average 6.31 versus 6.18, respectively, $P < 0.001$).

3.3. Surface water

Surface water NH₄⁺ concentrations decreased significantly in all treatments during the experiment ($P < 0.001$), with a steeper decrease in the presence of plants ($P < 0.001$) (Fig. S3). After 37 days, NH₄⁺ reached near-zero values in most treatments. Shortly after the first N addition, NH₄⁺ concentrations peaked briefly for urea, NH₄NO₃ and *Azolla* litter treatments, but then decreased again. In all treatments, NH₄⁺ was removed from surface water, irrespective of the presence of plants. Surface water NO₃⁻ was depleted within 37 days ($P < 0.001$) regardless of plant presence ($P = 0.097$) or N treatment ($P = 0.861$) (Fig. S4). Similar to NH₄⁺, NO₃⁻ concentrations peaked briefly after N addition in treatments containing NO₃⁻ (KNO₃ and NH₄NO₃) and subsequently decreased to near-zero values. In unvegetated treatments, surface water P shows a 4 to 18 times increase in concentration, except for *Azolla* living, which remained stable (Fig. 6). In treatments with *T. latifolia*, P was depleted with the exception of *Azolla* litter and control treatments.

3.4. CH₄ emissions

The first 60 days of the experiment, CH₄ emissions were low in all treatments, ranging from 0 to 1.8 mg m⁻² day⁻¹ with an average of 0.1 mg m⁻² day⁻¹ (Fig. 7). After 60 days, CH₄ fluxes were 1.11 mg m⁻² h⁻¹ on average. Starting from day 60, treatments diverged, with increased CH₄ emissions in several unvegetated treatments ($P < 0.001$) for urea in pulses ($P < 0.001$), slow-release urea ($P = 0.004$), slow release KNO₃ ($P = 0.007$) and *Azolla* litter ($P < 0.001$). On the final measurement day (day 95), the highest CH₄ fluxes were measured in mesocosms without plants treated with *Azolla* litter (59.9, 16.1 and 0.7 mg m⁻² d⁻¹) and urea in pulses (43.8, 35.6 and 3.2 mg m⁻² d⁻¹), followed by slow-release urea (12.4, 11.2 and 0.3 mg m⁻² d⁻¹) and slow-release KNO₃ (30.4, 0.4 and

Fig. 5. NH_4^+ concentrations in pore water over time A) in unvegetated mesocosms and B) in mesocosms with *T. latifolia*. Error bars represent standard deviations.

$0.3 \text{ mg m}^{-2} \text{ d}^{-1}$). Meanwhile, vegetated mesocosms had zero emissions in these treatments, with the exception of two *Azolla* litter mesocosms with low emissions (0.9 and $0.07 \text{ mg m}^{-2} \text{ d}^{-1}$). Pore water CH_4 concentrations one day prior to harvesting show similar patterns, with enhanced CH_4 concentrations in unvegetated treatments (on average 2.92 nmol L^{-1} compared to 0.55 nmol L^{-1} in *T. latifolia*; $P = 0.005$) (Fig. S5). Throughout the experiment, 90.8% of mesocosms without plants had detectable CH_4 emissions ($> 0.06 \text{ mg m}^{-2} \text{ d}^{-1}$) compared to 22.1% of mesocosms with *T. latifolia*.

3.5. N_2O emissions

Overall, N_2O emissions were rarely observed and only occurred in treatments with slow-release KNO_3 , KNO_3 in pulses and NH_4NO_3 in pulses, irrespective of plant presence (Fig. 8). On all measuring days the majority of fluxes were below detection limit ($< 1.88 \text{ mg m}^{-2} \text{ d}^{-1}$) and even after longer measurements (600 s) no fluxes were detected. Variation in N_2O fluxes was often high within treatments on the same measuring day. In slow-release KNO_3 treatments, N_2O emissions were observed for up to 20 days after the N application: twice in absence of plants and once in presence of plants. These N_2O emission events coincided with increased NO_3^- values in pore water. For pulses of KNO_3 and NH_4NO_3 , the overall trend shows enhanced emissions for 2 weeks after each N pulse, with a subsequent decrease to zero. Interestingly, this decrease appears to happen faster in mesocosms with plants, which had lower NO_3^- values in pore water compared to treatments without plants. Due to the extremely high number of zeros in this dataset, no reliable statistical model could be fit, thus we present only trends.

4. Discussion

The present study investigated the influence of *T. latifolia* and

surface water N composition on CH_4 and N_2O emissions and nutrient cycling after peat rewetting, to assess nutrient dynamics and GHG emissions of paludiculture with *T. latifolia* compared to an unvegetated state. An N load led to increased production of *T. latifolia* and promoted plant uptake of N and P from surface water, pore water and soil. Moreover, *T. latifolia* strongly reduced CH_4 emissions compared to an unvegetated state. N_2O emissions occurred episodically only after loading with NO_3^- .

4.1. *T. latifolia* effectively takes up N in various forms

The positive effect of N loading on length and biomass production of *T. latifolia* confirms the expected N limitation for growth on the nutrient-poor peat soil used in our experimental setting. This is confirmed by low plant N concentrations and N:P ratios as well as low N concentrations in water and soil, even after N addition. Steady state and pulsing flow regime had a similar enhancing effect on the performance of *T. latifolia*, which suggests efficient N uptake by *T. latifolia* after a pulse. The rapid loss of NO_3^- from pore water and surface water after loading in unvegetated mesocosms suggests high denitrification rates triggered by NO_3^- availability, which may have lowered plant-available N in NO_3^- treatments. The depletion of NO_3^- by plant uptake is expected to be positively correlated to plant biomass: in a pilot study in 2016 (similar set-up; unpublished results), weekly N pulses were more rapidly depleted when plants were present, and even more rapidly when plants were larger in size. Plants and improved biomass production may therefore provide a water purification function in paludiculture fields with frequent inflow (loading) of nutrient rich water. In the present study however, pore water samples were always taken one week after N addition, which overlooked short time uptake and speed of N loss in vegetated versus unvegetated mesocosms. Measurements of N in pore and surface water indicate that slow-release granules delivered

Fig. 6. Total P concentrations in surface water over time A) in unvegetated mesocosms and B) in mesocosms with *T. latifolia*. Error bars represent standard deviations.

N at a higher rate than expected based on supplier information. Exposure of slow-release granules to an aqueous medium may have caused elevated N concentrations in the initial phase of the experiment in all N-treatments, which was confirmed by incubating these granules in water and measuring N concentrations over time (results not shown). As plants were still small at the addition of the first N load, a large proportion of NO_3^- from pulse as well as slow-release treatments may have been lost as N_2 .

As opposed to NO_3^- , NH_4^+ was present in the pore water for a longer time span after N loading, and therefore remained available for plant uptake. This can explain the higher biomass yield in the urea pulse treatment compared to KNO_3 treatments. Furthermore, previous studies show that *T. latifolia* has a higher uptake affinity for NH_4^+ than for NO_3^- , with an optimum NH_4^+ uptake at a pH of 6.5, similar to our experiment (Brix et al., 2002). NH_4^+ toxicity through acidification in the root zone appears to be countered by *T. latifolia*, and higher pore water pH in vegetated treatments indicated soil buffering by the plant. The addition of *Azolla* did not improve plant performance compared to the control. Decomposition of *Azolla* may have been slow, resulting in lower plant available N throughout the experiment. Moreover, N removal by denitrification may have occurred with organic matter from *Azolla* as a carbon substrate. A higher N content of *T. latifolia* in *Azolla* treatments compared to the control does indicate an increase in N availability in both alive and litter treatments; however, we expect that the total N load in *Azolla* was lower than in mineral N treatments, leading to a diminished effect on plant growth.

4.2. *T. latifolia* paludiculture effectively improves water quality

When envisioning a sustainable production in paludiculture, sufficient nutrient provision to paludicrops is imperative. On top of this, nutrient removal from eutrophic water (e.g. agricultural runoff) can be an important ecosystem service of paludiculture. Our findings suggest that the inlet of N-rich water does not only improve biomass yield but also the uptake of N and P from surface water and soil. Plant N content and N:P ratios suggest ongoing N deficiency, which can be related to the addition of surplus P and K. Overall, N losses were substantial in both unvegetated and vegetated mesocosms. N in surface water and pore water was completely depleted in vegetated mesocosms, whereas NH_4^+ remained for a longer time in unvegetated treatments. This suggests that plant uptake occurs faster than microbial breakdown. Estimated N losses in unvegetated mesocosms were high (91–99%), suggesting effective N removal by gaseous losses also in the absence of plants. These losses may be caused by denitrification of NO_3^- to N_2 or N_2O and the oxidation of NH_4^+ by O_2 or ferric iron (Fe^{3+}) followed by denitrification. We expect that the decrease of NH_4^+ is mainly explained by nitrification, and that the role of NH_3 volatilization is negligible, as soil pH is below 8.0 (Vymazal, 2007). The relative importance of N removal by denitrification and by plant uptake is dependent on N load, with denitrification becoming the major process at high N loads if electron donor availability is not limiting (Bachand and Horne, 2000; Borin and Salvato, 2012; Burgin and Hamilton, 2007). Results of this study suggest that intermediate N pulses (e.g. through periodical loading with N-rich water) can be removed efficiently, especially when denitrification is complemented with plant N uptake. A constant inflow might not be necessary during the growing season.

Fig. 7. CH_4 emissions during the experiment in different N treatments A) without and B) with *T. latifolia*. Vertical dashed lines represent moments of N addition. Error bars depict standard deviations.

Several factors complicated our N budget calculation, which may cause an unbalanced estimate of N losses. N content at the start of the experiment was assessed only in one bulk sample. Although the peat was thoroughly mixed before addition to mesocosms, this may have neglected soil heterogeneity. Also, neither extent nor composition of gaseous losses were measured. Thus, N loss in vegetated mesocosms may be higher than estimated as a result of enhanced peat decomposition through root O_2 supply. Furthermore, pore water samples only assessed the top 10 cm of the soil, neglecting nutrient accumulation deeper in the (anoxic) peat.

Whereas N removal from surface water is mainly a microbial process, P removal is driven by plant biomass production. P added in excess was effectively removed from pore water and surface water by *T. latifolia*, especially when mineral N was supplied. This implies that *T. latifolia* can successfully mitigate P mobilisation in rewetted peat compared to an unvegetated state. Supply of N rich surface water can also be beneficial if P extraction from former agricultural soils is desired. To prevent nutrient accumulation and eutrophication in paludiculture, biomass harvesting is crucial.

4.3. CH_4 emissions fuelled by C, negated by *T. latifolia*

We found that CH_4 emissions were mostly low or absent, especially in *T. latifolia* treatments, despite anoxic conditions as indicated by the high N losses and the lack of nitrate. Average detected CH_4 fluxes of $1.11 \text{ mg m}^{-2} \text{ h}^{-1}$ were similar to values found in other mesocosm

studies (Bhullar et al., 2013; Green and Baird, 2012; Harpenslager et al., 2015; Karki et al., 2015). Substantial CH_4 release ($> 10 \text{ mg CH}_4 \text{ m}^{-2} \text{ d}^{-1}$) was only observed in four treatments after 60 days. Extreme values (up to $59.9 \text{ mg m}^{-2} \text{ d}^{-1}$) were comparable to rewetted bare former agricultural top soils, flooded fertilized rice paddies or FSW constructed wetlands (Harpenslager et al., 2015; Lindau et al., 1991; Mander et al., 2014b). The 60-day lag phase can be attributed to several factors. First of all, the (re-)establishment of methanogenic archaea requires time; this can take several years when peat has been exposed to oxic conditions (Juottonen et al., 2012). Moreover, the supply of substrate (acetate and/or H_2) by decomposition and mineralisation is assumed to be slow, as the used peat originates from a deep recalcitrant layer. However, the increase in pH after liming is expected to counter these effects and enhance decomposition and mineralisation (Van Diggelen et al., 2015). Additionally, the CH_4 production potential of *Sphagnum* peat has been shown to increase after raising the pH (Smolders et al., 2002; Tomassen et al., 2003).

In treatments loaded with NO_3^- , low CH_4 production in the initial 60 days can be explained by the prevalence of denitrification – a thermodynamically more favourable process than methanogenesis, with denitrifiers outcompeting methanogens for organic substrate (Achnich et al., 1995). Additionally, reduction of NO_3^- can be coupled to CH_4 oxidation in which NO_3^- is the terminal electron acceptor (Bodelier and Laanbroek, 2004; Haroon et al., 2013; Raghoebarsing et al., 2006; Zhu et al., 2012), further reducing CH_4 emissions until NO_3^- is depleted. In NH_4^+ loaded treatments, NH_4^+ present in pore water can reduce CH_4

Fig. 8. N₂O emission rates during the experiment at different N treatments A) without and B) with *T. latifolia*. Only treatments in N₂O emissions were detected are presented. Vertical dashed lines represent moments of N addition.

emissions when methane oxidation is N limited, as has been shown in rice paddies (Bodelier et al., 2000; Bodelier and Laanbroek, 2004). In this study, however, it is not N but C that seems to be limiting for methanogenesis: *Azolla* litter as well as urea, the predominant treatments resulting in considerable CH₄ emissions, contain carbon that can only become available over time through breakdown. This labile carbon can provide acetate for methanogens, promoting CH₄ production. Our finding of C substrate limited methanogenesis corroborates with previous studies in bogs and fen peat cores (Bhullar et al., 2013; Smolders et al., 2002), flooded rice paddies (Lindau et al., 1991; Wang et al., 2017), and in FWS constructed wetlands (Mander et al., 2014b).

Given this important role of the substrate availability on methanogenesis, we would expect *T. latifolia* to have a significant fuelling effect on CH₄ production, especially in treatments where biomass production was further stimulated by increased N loads (Chanton et al., 1993; Couwenberg and Fritz, 2012; Ding et al., 2004). However, in *T. latifolia* treatments, CH₄ emissions and pore water concentrations remained low regardless of N or C loading. Rhizospheric CH₄ oxidation through O₂ input by *T. latifolia* (Jespersen et al., 1998) therefore appears to overrule potential stimulation of methanogenesis by the provision of labile carbon. It is likely that plant-mediated CH₄ transport was irrelevant in our experiment, as pore water CH₄ concentrations were low. A similar effect of vegetation was found in FWS constructed wetlands and created riverine wetlands (Mander et al., 2014b). *T. latifolia* would therefore be a highly suitable paludiculture crop considering GHG mitigation, in contrast to CH₄-fuelling plant species such as reed canary grass (*Phalaris arundinacea*) (Karki et al., 2015) and sedges (*Carex* and *Eriophora* species) (Green and Baird, 2012; Günther et al., 2015; Koelbener et al., 2010).

In our experiment, plant litter was close to absent. When *T. latifolia*

is cultivated on a longer term, accumulation of organic carbon from degrading roots and shoots may fuel methanogenesis (Mander et al., 2014b). This can be prevented by harvesting of aboveground biomass, which is common practice in paludiculture, more so as remaining *T. latifolia* stubbles appear to have no effect on CH₄ emissions (Günther et al., 2015). Furthermore, harvesting adds the possibility to extract nutrients from the peatland soil and water (Schulz et al., 2011) and harvested biomass provides an economic incentive (Wichtmann et al., 2016). Besides harvesting practices, environmental conditions such as changes in water table, composition of incoming water, temperature, light regime and vegetation development can cause seasonal variation in CH₄ efflux (Christensen et al., 2003).

4.4. N₂O emission insignificant

Incidental N₂O emissions in KNO₃ treatments may be explained by high NO₃⁻ concentrations combined with low availability of organic carbon substrate, resulting in incomplete denitrification (Wunderlin et al., 2012). N₂O emissions after NO₃⁻ loading irrespective of plant presence correspond to Karki et al. (2015), where N loading induced N₂O emissions but plants only had a minor abating effect. In treatments with *T. latifolia*, a faster decrease in N₂O emissions can be expected as a result of NO₃⁻ uptake by the plants (Mander et al., 2014b). Raising the pH in our soil by liming may have had a diminishing effect on N₂O emission, which is negatively correlated with pH (Van Den Heuvel et al., 2011).

4.5. Conclusions and recommendations

Our study shows that paludiculture with *T. latifolia* provides

opportunities for water purification and that the inlet of N rich surface water stimulates biomass production. Next to realizing C sequestration in biomass, *T. latifolia* can efficiently remove N and P from surface water and pore water and thus prevent nutrient mobilisation and accumulation of P and NH_4^+ , in contrast to unvegetated controls. On top of this, *T. latifolia* suppresses CH_4 emissions, which can be induced by N loading in an unvegetated state, and more so when organic C is provided next to N. These findings show that the cultivation of *T. latifolia* after rewetting has promising perspectives for the restoration of ecosystem services in drained peatlands. Paludiculture with *T. latifolia* can be used for the purification of nutrient-rich water while keeping CH_4 and N_2O emissions at a minimum. This nutrient supply can enhance biomass production and yields and thus provide an economic incentive. Low and infrequent N_2O emissions even after an N pulse further support the possibility for paludiculture to receive and purify N-rich surface water. As the accumulation of labile carbon can promote methanogenesis in unvegetated soils, chemical composition as well as flow regime of incoming water should be optimized and related to growing season. Harvesting of aboveground biomass (i.e. paludiculture) is essential to reduce accumulation of organic matter and nutrients.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ecoleng.2018.09.008>.

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