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# Nitrogen Removal in a Small Constructed Wetland: An Isotope Mass Balance Approach

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The nitrogen (N) removal potential of constructed wetlands is increasingly used to lower the N load from agricultural nonpoint sources to inland and coastal waters. To determine the removal efficiency and key factors limiting wetland N removal, N fluxes were studied in a small constructed wetland in Central Switzerland. With an isotope mass balance approach integrating the natural isotope signature of nitrate ( $\text{NO}_3^-$ ), ammonium ( $\text{NH}_4^+$ ), and particulate nitrogen (PN), the N transformations such as assimilation, mineralization, nitrification, and denitrification were quantified. On average, the wetland removed  $45 \text{ g m}^{-2} \text{ yr}^{-1}$  N during the studied 2.5 years, corresponding to a removal efficiency of 27%. Denitrification contributed 94% to the N removal, while only 6% of the removed N accumulated in the sediments. Denitrification was most efficient during periods with an oxic water column overlying anoxic sediments, as  $\text{NH}_4^+$  released during mineralization of sediment organic matter was completely nitrified and subsequently denitrified at the sediment–water interface. During water column anoxia,  $\text{NH}_4^+$  accumulated in the water and fueled assimilation by duckweed and internal recycling. The  $\text{NO}_3^-$ -N isotope signature in the wetland mainly reflected the mineralization of sediment organic matter and subsequent nitrification, while denitrification at the sediment–water interface produced no fractionation.

## Introduction

Wastewater discharge and the application of inorganic N fertilizer and liquid manure in excess of plant demands augment the N transfer to aquatic ecosystems worldwide (1, 2). Concerns over coastal eutrophication (3) has triggered political response such as the recommendation of the Paris Commission to reduce N inputs to the tributaries of the North East Atlantic by 50% (4). Improvements in wastewater treatment were achieved rapidly, but lowering agricultural nonpoint source pollution has proven to be more difficult and tedious.

Natural and constructed wetlands have the potential to decrease the agricultural N discharge (5). Thus, recently in many countries in Europe and the United States, wetlands, riparian buffer strips, and ponds have been restored or artificially constructed at the interface between cultivated land and receiving waters. Fed with drainage water, storm-

water, or river water, these mostly small, low-maintenance “treatment plants” remove part of their N load as they trap particulate matter, produce and accumulate biomass, and eliminate N by denitrification (6). To which degree individual N transformations contribute to N removal and which factors limit these transformations are key questions for improving the removal efficiency of wetlands.

The isotopic signature of N species reflects their origin (7) and allows the study of N processing. Microbial N transformations discriminate against the heavy N isotope, leaving the substrate  $^{15}\text{N}$  enriched and the product  $^{15}\text{N}$  depleted. In field studies determining denitrification in riparian buffer zones, decreasing  $\text{NO}_3^-$  concentrations have been successfully linked to an increasing accumulation of  $^{15}\text{N}$  in  $\text{NO}_3^-$  (8). In wetlands, however, the quantitative assessment of denitrification based on simple measurements of the isotopic  $\text{NO}_3^-$  composition has mostly failed, because additional fractionating processes were neglected or could not be assessed (9).

In the present work, we studied the fluxes of all significant N species entering and leaving a small constructed wetland in Central Switzerland and investigated the natural isotopic composition not only of  $\text{NO}_3^-$ , but also of  $\text{NH}_4^+$ , of the biomass, and of the sediment particulate nitrogen in the wetland. Our objectives were (i) to investigate whether the relevant N transformations, in particular denitrification, nitrification, and mineralization, can be quantified with an isotope mass balance approach; (ii) to assess the relative importance of the processes responsible for the long-term N removal, and (iii) to identify key factors controlling the N transformations in wetlands.

## Materials and Methods

**Study Site.** Wetland “Boden” is located in the Central Swiss Plateau at 540 m above sea level. Precipitation averages  $1250 \text{ mm yr}^{-1}$ , and the mean annual temperature is approximately  $10^\circ\text{C}$ . The predominantly loamy, water-logged soil of the watershed is drained with subsurface drainage pipes and cultivated as grassland. From the end of February to mid-November, liquid manure is frequently spread on the grassland.

In winter 2001 and 2002, the surface-flow wetland was constructed at the interface between two subsurface drainage systems and the small receiving river “Waldbach”, a tributary of Lake Sempach. The wetland’s watershed spans 8.4 ha of slightly sloped grassland. The wetland comprises a total water surface area of  $720 \text{ m}^2$  with a volume of  $700 \text{ m}^3$  and a maximum depth of 3 m. Three separate basins were excavated, two small and flat sedimentation basins ( $20 \text{ m}^3$  and  $80 \text{ m}^3$ ) and one deep pond ( $600 \text{ m}^3$ ) (for a map, see Supporting Information). Both sedimentation basins, each fed by a separate drainage pipe (inlets I and II), discharge into the main pond. Inlet II delivers only soil drainage water, whereas inlet I additionally discharges surface water from the nearby farm yard. The wetland’s outlet pipe is embedded in the surrounding dam 0.6 m below its top edge. In summer 2003 and 2004 the water surface was covered with duckweed (*Lemna minor* L.).

**Hydrological Monitoring.** From May 2002 to October 2004, the water discharge at inlet I and the outlet was measured with gaged V-notch weirs (ATM/N water level gage, STS, Sirmach, Switzerland). The water discharge of inlet II was measured with an inductive flow meter (Promag 53W, Endress+Hauser, Reinach, Switzerland). In the main pond the water level was monitored with an ATM/N gage. Readings of flow rate and water level were recorded every 10 min with

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a CR10 data logger (Campbell Scientific, Logan, UT). Every 20 min, water temperature was measured 15 cm below the water surface (Minilog12, VEMCO, Shad Bay, Canada).

**Sampling.** In 2002, water samples of inlet I, inlet II, and the outlet were collected manually every second day. In 2003 and 2004, two automatic samplers (6712, ISCO, Lincoln, NE; Manning 4900, TN Technologies, Round Rock, TX) sampled inlet I and the outlet whenever distinct amounts of water (inlet I: 4.3 m<sup>3</sup>; outlet: 8.6 m<sup>3</sup>) had passed through. A composite sample was made from 20 and 10 spot samples at the inlet I and the outlet, respectively. As inlet II contributed only 13% to the total water load (see below), its sampling was discontinued in 2003, but restarted in 2004 for isotope analysis. For 2003, its N load was estimated from the measured water discharge in 2003 and median concentrations of the different N species observed in 2002 and 2004.

To study the nitrogen isotopic signature, inlet I, inlet II and the outlet were sampled manually at least once per month from March to October 2004. In addition, monthly samples of the water column, the duckweed and the sediment trap (radius: 7.1 cm, height: 50 cm) installed at the deepest site of the main pond were collected. Concurrently, oxygen and temperature profiles were measured in situ at approximately 10 a.m. (CellOx325, WTW, Weilheim, Germany). From the heat balance of the wetland the turbulent diffusion coefficient (10) was calculated. Sediment cores were collected in March 2003 and November 2004. Sediment and rinsed duckweed were freeze-dried and ground.

**Chemical Analysis.** Water samples were filtered through 0.45-μm cellulose acetate filters and stored at 5 °C until analysis. NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> concentrations were determined with standard photometric techniques (11). Total nitrogen (TN) and dissolved nitrogen (DN) of the water samples and TN of duckweed and sediment samples were measured after alkaline persulfate digestion (12). Particulate nitrogen was calculated as the difference between TN and DN. Dissolved (mainly) organic nitrogen (DON) resulted from the subtraction of NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> from DN. For mass balance considerations PN was subdivided into allochthonous PN (PN<sub>al</sub>) and into autochthonous PN (PN<sub>au</sub>). All concentrations are given as g N m<sup>-3</sup>.

Nitrogen isotope ratios of samples are reported in standard δ notation (‰) relative to atmospheric N<sub>2</sub>. The degree of fractionation during a reaction is expressed as enrichment factor ε (‰), which can be approximated as the difference of the δ<sup>15</sup>N of the product and the δ<sup>15</sup>N of the substrate (13). For isotope analysis of <sup>15</sup>N<sub>NO3</sub> and <sup>15</sup>N<sub>NH4</sub>, samples were prepared according to Silva et al. (14) and Lehmann et al. (15), respectively. NO<sub>3</sub><sup>-</sup> was collected on anion-exchange resins (AG 1-X8, 100–200 mesh size; Bio-Rad, Hercules, CA), extracted afterward and precipitated as AgNO<sub>3</sub>. NH<sub>4</sub><sup>+</sup> was collected on cation-exchange resins (AG 50W-X8, 100–200 mesh size, Bio-Rad, Hercules, CA), which were subsequently dried, ground and homogenized. AgNO<sub>3</sub>, NH<sub>4</sub><sup>+</sup>-containing resin, samples of duckweed, sediment trap material, and sediment were combusted in an elemental analyzer (CNS 2500, Carlo Erba, Milan, Italy) and continuously transferred into an isotope ratio mass spectrometer (IsoPrime Stable Isotope Ratio MS, GV instruments, Manchester, UK). The reproducibility of the δ<sup>15</sup>N measurement was ±0.2‰.

**Mass Balance Calculations.** Denitrification (deni) and nitrification (nitr) were calculated combining the NO<sub>3</sub><sup>-</sup> mass balance (eq 1) and the mass balance of its isotopic composition (eq 2). The change of the NO<sub>3</sub><sup>-</sup> stock in the wetland's water column during the investigated period (dNO<sub>3</sub><sup>-</sup>/dt) equals the difference between NO<sub>3</sub><sup>-</sup> sources, i.e., NO<sub>3</sub><sup>-</sup> input (in) and nitrification, and NO<sub>3</sub><sup>-</sup> sinks, i.e., NO<sub>3</sub><sup>-</sup> output (out),

**TABLE 1. Nitrogen Balance of Wetland Boden (05/2002–10/2004)**

	input (kg)	output (kg)	removal (%)
TN	301	220	27
NO <sub>3</sub> <sup>-</sup> -N	143	101	29
NH <sub>4</sub> <sup>+</sup> -N	15	16	-1
DON	100	78	22
PN	43	25	42

denitrification, and uptake of NO<sub>3</sub><sup>-</sup> into PN<sub>au</sub> (up).

$$\frac{d\text{NO}_3^-}{dt} = \text{in} + \text{nitr} - \text{out} - \text{deni} - \text{up} \quad (1)$$

NO<sub>3</sub><sup>-</sup> fluxes at the inlet and outlet were calculated by multiplying the average time or flow proportional NO<sub>3</sub><sup>-</sup> concentrations by the water discharge integrated over the corresponding period. Uptake of NO<sub>3</sub><sup>-</sup> into PN<sub>au</sub> includes production of plankton PN<sub>plankton</sub>, duckweed PN<sub>duckweed</sub>, and autochthonous sediment trap material PN<sub>trap,au</sub>. Allochthonous PN was assumed to settle completely within the wetland. N<sub>2</sub>O production during nitrification and denitrification was disregarded in the mass balance as it usually amounts to less than 0.5% of the N<sub>2</sub> flux (16).

Analogous to eq 1, the mass balance for the isotopic composition of NO<sub>3</sub><sup>-</sup> was calculated as

$$\frac{d\text{NO}_3^- \cdot \delta^{15}\text{N}_{\text{NO}_3}}{dt} = \text{in} \cdot \delta^{15}\text{N}_{\text{NO}_3, \text{in}} + \text{nitr} \cdot \delta^{15}\text{N}_{\text{NO}_3, \text{nitr}} - \text{out} \cdot \delta^{15}\text{N}_{\text{NO}_3, \text{out}} - \text{deni} \cdot \delta^{15}\text{N}_{\text{N}_2} - \text{up} \cdot \delta^{15}\text{N}_{\text{PN}_{\text{au}}} \quad (2)$$

The δ<sup>15</sup>N measured for duckweed was also applied to plankton, whose isotopic composition was not analytically determined. For δ<sup>15</sup>N<sub>N2</sub> and δ<sup>15</sup>N<sub>NO3, nitr</sub> see discussion.

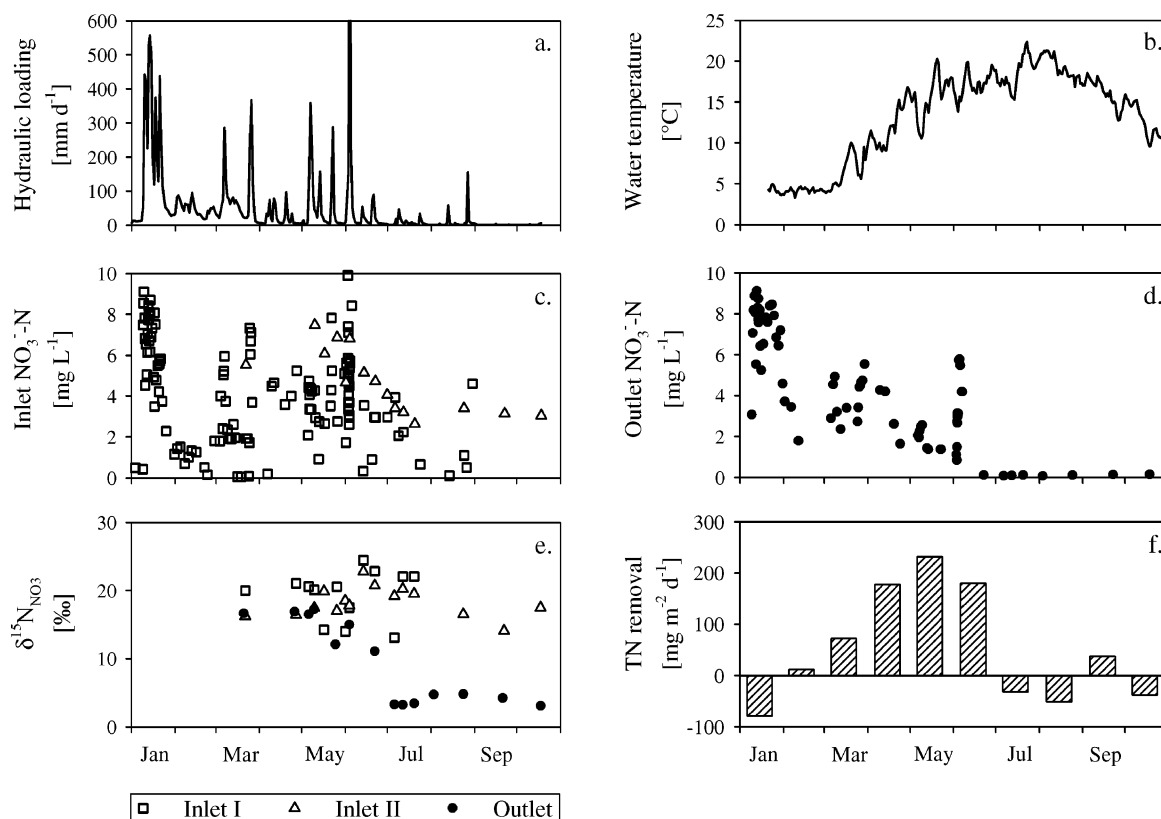
**Laboratory Experiments.** Potential denitrification was measured in duplicate laboratory experiments recording the NO<sub>3</sub><sup>-</sup> decrease from the water overlying two undisturbed sediment cores (radius: 2.9 cm, water column height: 21 cm). In three successive series, NO<sub>3</sub><sup>-</sup> was added to the stirred water column to a final concentration of approximately 1.90 mg L<sup>-1</sup>, 4.98 mg L<sup>-1</sup> and 15.60 mg L<sup>-1</sup>. Sediment cores were incubated at 9 °C in the dark and sampled after 6, 20, 30, 44 h and subsequently every 24 h following each spike.

## Results

**Hydrologic Regime.** Average hydraulic loading based on the wetland's surface area was 150 mm d<sup>-1</sup> in 2002, but only 50 mm d<sup>-1</sup> in 2003 and 2004 due to exceptionally dry weather. Inlet I contributed 87% and inlet II 13% to the total water discharge. The outlet pipe discharged 81% of the water from the wetland, while 19% was lost as downward seepage.

**Nitrogen Balance.** Average TN input to the wetland was 167 g m<sup>-2</sup> yr<sup>-1</sup> with respect to the wetland surface. Nitrate was the main N species in the drainage inlets of the wetland and contributed 48% of the TN load (Table 1). Median NO<sub>3</sub><sup>-</sup>-N concentration of inlet I and inlet II were 1.91 mg L<sup>-1</sup> and 3.04 mg L<sup>-1</sup>, respectively. Up to 12.9 mg L<sup>-1</sup> were detected in both inlets during the first intense rainfalls after the long dry period in summer 2003. During 2004, the NO<sub>3</sub><sup>-</sup> loading, calculated from the water discharge (Figure 1a) and the NO<sub>3</sub><sup>-</sup> concentration (Figure 1c), was high from January to June (292 mg m<sup>-2</sup> d<sup>-1</sup>), but decreased subsequently due to the dry weather from July to October (13 mg m<sup>-2</sup> d<sup>-1</sup>) (Table 2). δ<sup>15</sup>N<sub>NO3</sub> of inlets I and II ranged from 13.1 to 24.4‰ and were typical for manure-derived δ<sup>15</sup>N<sub>NO3</sub> (17); δ<sup>15</sup>N<sub>NO3</sub> of the outlet ranged from 3.1 to 16.9‰ (Figure 1e).

Surface specific removal was 45 g m<sup>-2</sup> yr<sup>-1</sup>, corresponding to a removal efficiency of 27% (Table 1). The PN and NO<sub>3</sub><sup>-</sup>



**FIGURE 1.** Hydraulic loading rate (a), average daily water temperature (b), NO<sub>3</sub><sup>-</sup> concentration of inlets I and II (c) and the outlet (d), δ<sup>15</sup>N<sub>NO3</sub> of inlet I, inlet II, and the outlet (e), and TN removal of Wetland Boden (f) from January to October 2004.

**TABLE 2. Nitrogen Fluxes (mg m<sup>-2</sup> d<sup>-1</sup>) in Wetland Boden (April–October 2004)**

		mg m <sup>-2</sup> d <sup>-1</sup>						
		Apr 03/22–04/27	May 04/27–05/26	Jun 05/26–06/22	Jul 06/22–07/20	Aug 07/20–08/24	Sep 08/24–09/22	Oct 09/22–10/18
NO <sub>3</sub> <sup>-</sup> input	in	250	266	374	20	5	22	3
NO <sub>3</sub> <sup>-</sup> output	out	163	104	240	27	1	1	1
change of the NO <sub>3</sub> <sup>-</sup> stock	dNO <sub>3</sub> <sup>-</sup> /dt	-31	-48	-14	-8	-1	0	-1
NO <sub>3</sub> <sup>-</sup> uptake into PN <sub>au</sub>	up							
plankton	PN <sub>plankton</sub>	8	13	108	-11	1	7	-1
duckweed	PN <sub>duckweed</sub>	0	15	54	15	-3	-75	0
sediment trap <sup>a</sup>	PN <sub>trap,au</sub>	0	61	77	116	51	199	56
sediment trap, allochthonous	PN <sub>trap,al</sub>	26	27	112	10	9	9	0
denitrification <sup>b</sup>	deni	129	222	119	1	6	5	4
nitrification <sup>b</sup>	nitr	19	101	210	120	50	115	56
nitrification supplied with NH <sub>4</sub> <sup>+</sup> from								
mineralized DON <sup>c</sup>	nitr <sub>DON</sub>	28	77	88	-13	17	-6	-14
allochthonous NH <sub>4</sub> <sup>+</sup> <sup>d</sup>	nitr <sub>NH4,al</sub>	-9	-19	-8	-44	-61	5	-14
benthic NH <sub>4</sub> <sup>+</sup> <sup>e</sup>	nitr <sub>NH4,benthic</sub>	0	43	130	177	94	116	84

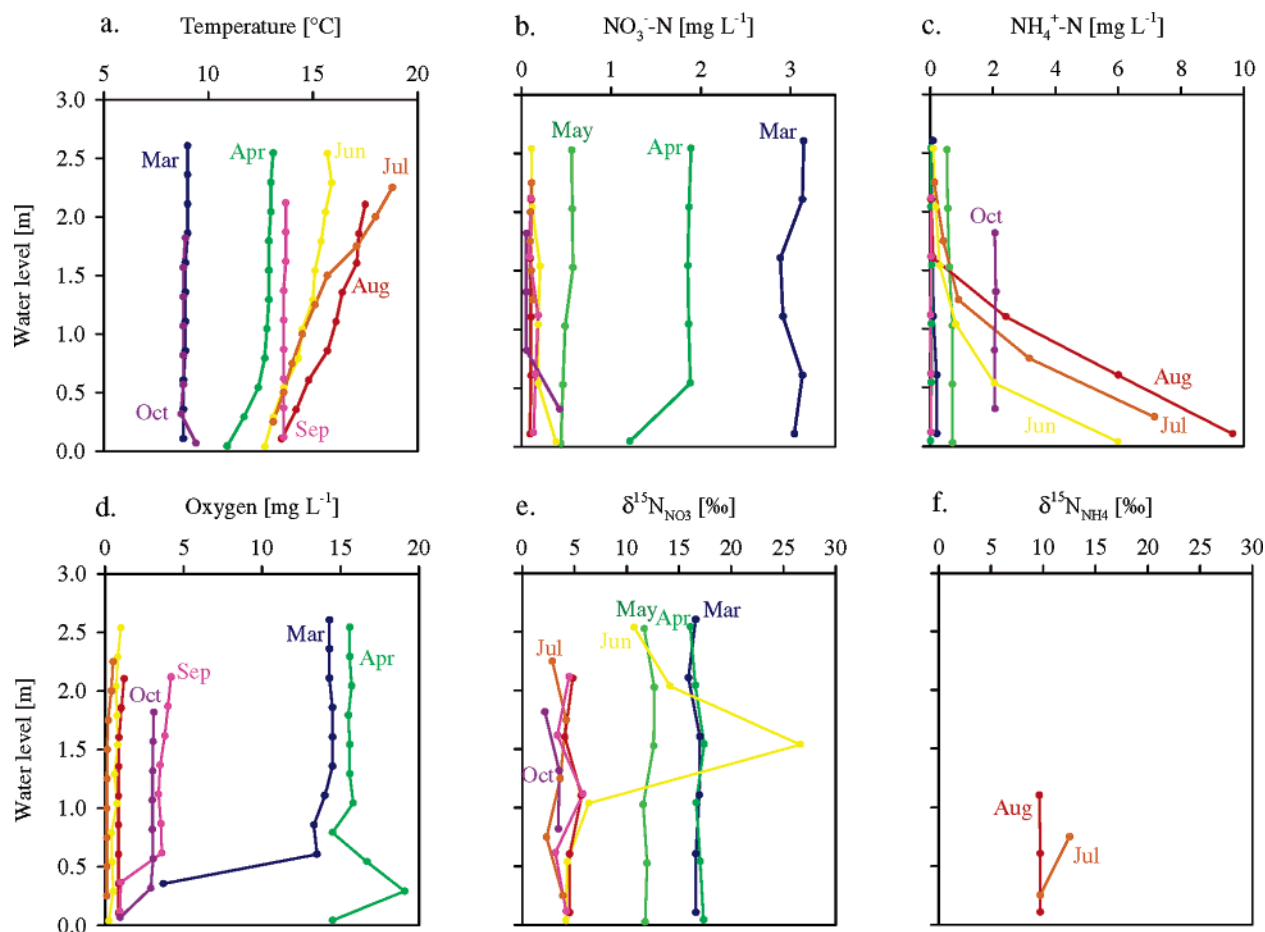
<sup>a</sup> Assuming complete settling of PN<sub>ai</sub> in the wetland (PN<sub>ai</sub> = PN<sub>trap,ai</sub>), PN<sub>trap,au</sub> was calculated from PN<sub>trap</sub> = PN<sub>trap,au</sub> + PN<sub>trap,al</sub>. <sup>b</sup> Calculated from eqs 1 and 2. <sup>c</sup> dDON/dt = in - out - nitr<sub>DON</sub>. <sup>d</sup> dNH<sub>4</sub>/dt = in - out - nitr<sub>NH4,al</sub>. <sup>e</sup> nitr = nitr<sub>DON</sub> + nitr<sub>NH4,al</sub> + nitr<sub>NH4,benthic</sub>.

load decreased by 42% and 29%, respectively, whereas the NH<sub>4</sub><sup>+</sup> output balanced the NH<sub>4</sub><sup>+</sup> input. TN removal varied seasonally and interannually: In 2002, 41% of the TN load, but in 2003 only 16% of the TN load, was removed in the wetland. In 2004, TN removal was -35 mg m<sup>-2</sup> d<sup>-1</sup> (-4%) in January and February, 164 mg m<sup>-2</sup> d<sup>-1</sup> (36%) from March to June, and -17 mg m<sup>-2</sup> d<sup>-1</sup> (-57%) from July to October (Figure 1f). Detailed N fluxes during the isotope investigation from April to October 2004 are shown in Table 2. Nitrogen removal exceeded phosphorus (P) removal 41 fold (18).

**Wetland Chemistry.** According to the temperature profiles (Figure 1b), the wetland's water column was completely mixed in spring and autumn 2004. During the low water discharge period from June to August, the mixing depth was limited to the upper 1 m. During turnover in April and May, O<sub>2</sub> concentration in the water column exceeded 13 mg L<sup>-1</sup>

due to intense photosynthesis; in June, it decreased to values below detection limit right above the sediment (Figure 2d). Nitrate concentration declined from approximately 3.00 mg L<sup>-1</sup> in March to 0.10 mg L<sup>-1</sup> in June (Figure 2b). Simultaneously, δ<sup>15</sup>N<sub>NO3</sub> decreased from 16.8‰ in March and April to 12.1‰ in May and averaged 3.9‰ after July (Figure 2e). Ammonium concentration was smaller than 0.10 mg L<sup>-1</sup> in March and April but was up to 10.0 mg L<sup>-1</sup> above the sediment from June to August (Figure 2c). δ<sup>15</sup>N<sub>NH4</sub> ranged from 9.7 to 12.5‰ (Figure 2f).

Duckweed covering the water surface from May to August (Figure 1b) reached its maximum density of 280 g dwt m<sup>-2</sup> in July. Its N content declined from 50.4 mg g<sup>-1</sup> dwt in May to 16.0 mg g<sup>-1</sup> dwt in August. Simultaneously, its δ<sup>15</sup>N decreased from 14.4‰ in June to 8.2‰ in August. Sedimentation rate in the wetland ranged from 4 to 22 g dwt m<sup>-2</sup> d<sup>-1</sup>.



**FIGURE 2.** Seasonal variation of the water column chemistry in Wetland Boden in 2004: Water temperature (a),  $\text{NO}_3^-$  concentration (b),  $\text{NH}_4^+$  concentration (c), oxygen concentration (d),  $\delta^{15}\text{N}_{\text{NO}_3}$  (e), and  $\delta^{15}\text{N}_{\text{NH}_4}$  (f) plotted versus depth.

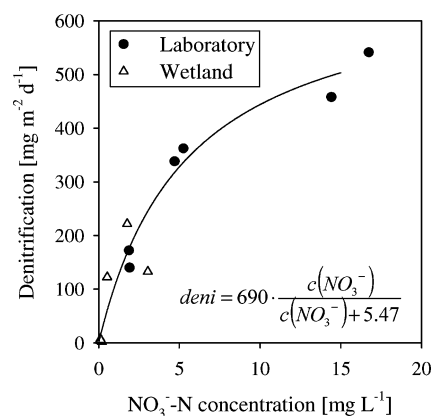
The sediment trap material had a minimum N content of  $6.4 \text{ mg g}^{-1}$  dwt in March and a maximum N content of  $17.1 \text{ mg g}^{-1}$  dwt in August.  $\delta^{15}\text{N}$  of the sediment trap material decreased from  $10.2\text{‰}$  in April to  $5.3\text{‰}$  in December. At the bottom of the main pond, a sediment layer with a mean N content of  $2.5 \text{ mg g}^{-1}$  dwt and a  $\delta^{15}\text{N}$  of  $5.5\text{‰}$  ( $\pm 0.2$ ) accumulated during the 2.5 years. The  $\delta^{15}\text{N}$  of the sediment collected in the sedimentation pond at inlet I ( $5.1\text{‰} \pm 0.3$ ) reflected the  $\delta^{15}\text{N}$  of allochthonous PN. The N:P ratio of the wetland's sediment was 2.3.

**Laboratory Experiments.** Denitrification rates followed a Michaelis–Menten equation relative to the  $\text{NO}_3^-$  concentration ( $r^2$ : 0.961). They increased in proportion to the  $\text{NO}_3^-$  concentration with an initial slope of  $0.13 \text{ m d}^{-1}$  and converged on a maximum of  $690 \text{ mg m}^{-2} \text{ d}^{-1}$  ( $\pm 70$ ). The half saturation constant was  $5.47 \text{ mg L}^{-1}$  (Figure 3).

## Discussion

**Nitrogen Removal.** The TN removal of  $45 \text{ g m}^{-2} \text{ yr}^{-1}$  (27%) observed in Wetland Boden is within the range reported for wetlands (19) and lakes (20) that reduce the N load from nonpoint source pollution by an average of 27% (see Supporting Information). The removed N is either accumulated in the sediments or eliminated by denitrification, whereas P removal is only due to sediment accumulation. Given that 41 times more N than P was removed in Wetland Boden, but only 2.3 times more N than P accumulated in the sediment, only 6% ( $3 \text{ g m}^{-2} \text{ yr}^{-1}$ ) of the removed N was stored in the sediment, whereas 94% ( $42 \text{ g m}^{-2} \text{ yr}^{-1}$ ) was denitrified and lost to the atmosphere.

TN removal in Wetland Boden was not constant throughout the studied period as shown in Figure 1f. Low water

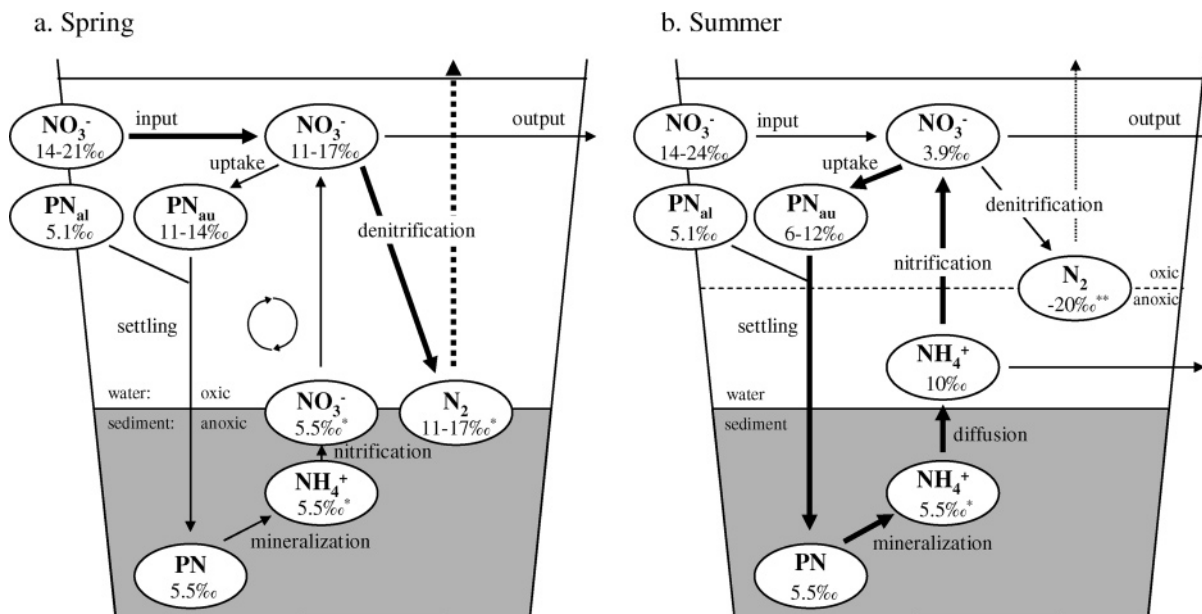


**FIGURE 3.** Denitrification as determined in laboratory experiments (filled circles) and calculated for Wetland Boden according to the isotope mass balance approach (open triangles) plotted versus  $\text{NO}_3^-$  concentration.

temperature and short water residence time limiting microbial activity and plankton assimilation accounted for the low or even negative TN removal during winter 2004 but could not explain the decline of the absolute and percentage TN removal during summer. Therefore, N transformations were quantified monthly with the isotope mass balance discussed below.

**Nitrate Isotope Signature To Identify Nitrogen Transformations.** Denitrifying bacteria discriminate against the heavier nitrogen isotope, if a sufficient amount of  $\text{NO}_3^-$  is available. Thus, during denitrification the microbially produced  $\text{N}_2$  is depleted of  $^{15}\text{N}$ , whereas the remaining  $\text{NO}_3^-$





**FIGURE 4.** Isotope mass balance model for N removal and recycling in Wetland Boden. N fluxes (arrows), N reservoirs (ovals), and their isotopic signature (‰) as measured or estimated (\*) in Wetland Boden from March to June (a) and July to October (b), 2004. (\*\*For water column denitrification a fractionation factor of  $-24\text{‰}$  was assumed.)

becomes enriched with  $^{15}\text{N}$ . Fractionation observed in wetlands ( $\epsilon$ :  $-2.5\text{‰}$ ), however, was far lower than determined in laboratory experiments ( $\epsilon$ :  $-17$  to  $-29\text{‰}$ ) (9). In Wetland Boden,  $\delta^{15}\text{N}_{\text{NO}_3}$  remained constant while the  $\text{NO}_3^-$  concentration decreased between March and April (Figure 2b, e). In addition,  $\delta^{15}\text{N}_{\text{NO}_3}$  decreased until June, and the outlet  $\delta^{15}\text{N}_{\text{NO}_3}$  did not exceed those of the inlets (Figure 1e).

Two factors summarized in Figure 4 could account for this pattern: (i) As denitrifying enzyme activity requires an  $\text{O}_2$  concentration below  $0.2 \text{ mg L}^{-1}$  (21) and the water column was oxic from March to May, denitrification in spring was limited to the anoxic sediment layer (Figure 4a). Benthic denitrification acts as a perfect sink since nitrate diffusing across the water–sediment interface is completely consumed irrespective of its isotopic composition (22, 23). Hence, denitrification during spring did not produce isotope fractionation. (ii) For the further decrease of  $\delta^{15}\text{N}_{\text{NO}_3}$  between May and July, an additional process yielding  $^{15}\text{N}$ -depleted  $\text{NO}_3^-$  is required. The fixation of atmospheric  $\text{N}_2$  ( $\delta^{15}\text{N}$ :  $0\text{‰}$ ) was of minor importance, as an investigation of the wetland's phytoplankton revealed. Therefore, the light  $\text{NO}_3^-$  most likely originated from  $\text{NH}_4^+$  nitrification. As the  $\text{NH}_4^+$  input via the inlets was negligible, additional  $\text{NH}_4^+$  must have been supplied by mineralization of sediment organic matter and DON (Table 2). Because benthic mineralization has been shown to lack fractionation (17, 22), the isotopic composition of  $\text{NH}_4^+$  released from the sediments was similar to the sediment PN (5.5‰). In spring, regardless of these  $\text{NH}_4^+$  supplies, the  $\text{NH}_4^+$  concentration in the water column remained low, indicating complete nitrification. Hence, the isotope signature of  $\text{NO}_3^-$  and the parent PN were identical (Figure 4a). In summer, in contrast, the average water column  $\delta^{15}\text{N}_{\text{NH}_4}$  of  $10\text{‰}$  and the  $\delta^{15}\text{N}_{\text{NO}_3}$  of  $3.9\text{‰}$  indicate that only part of the benthic  $\text{NH}_4^+$  was nitrified, resulting in heavier  $\text{NH}_4^+$  and lighter  $\text{NO}_3^-$  (Figure 4b).

When interpreting the isotopic signature of  $\text{NH}_4^+$ , it must be kept in mind that part of the DON from the drainage inlets may also have contributed to the production of  $\text{NH}_4^+$ . In spring DON input exceeded its output by 48%; in summer DON input and output were nearly balanced (Table 2). Considering the inlet DON as an intermediate product of allochthonous PN mineralization to  $\text{NH}_4^+$ ,  $\delta^{15}\text{N}_{\text{NH}_4}$  and  $\delta^{15}\text{N}_{\text{DON}}$  must be closely linked to the  $\delta^{15}\text{N}_{\text{PN,al}}$  of  $5.1\text{‰}$ . With

an enrichment factor of  $-2\text{‰}$  determined for PN-derived DON in Lake Michigan and Lake Superior (24),  $\delta^{15}\text{N}_{\text{DON}}$  and  $\delta^{15}\text{N}_{\text{NH}_4}$  in Wetland Boden would be in the range of, or below,  $3.1\text{‰}$ .

On the other hand, the  $\delta^{15}\text{N}$  of  $\text{NH}_4^+$  released during decomposition of autochthonous PN may have exceeded the average sediment  $\delta^{15}\text{N}$  of  $5.5\text{‰}$ , as the sediment composed of degraded material was significantly lighter than the duckweed ( $8.2$ – $14.4\text{‰}$ ) and the settling material ( $5.3$ – $10.2\text{‰}$ ) collected in the sediment trap. Part of this discrepancy can be explained by the settling of allochthonous PN ( $5.1\text{‰}$ ), which contributed 55% to the total PN deposition during spring 2004, but only 7% during low loading in summer. The accumulation of light PN in the sediments may as well be due to the preferential release of heavy N under anoxic conditions, which has been described in field and laboratory studies (25–27). These findings, which are in contrast to general expectations, were attributed to the selective decomposition of  $^{15}\text{N}$ -enriched PN (e.g., proteins) (25) or bacterial growth on the decomposing material (26, 27).

#### Quantitative Assessment of Nitrogen Transformations.

Solving eqs 1 and 2 with the isotope signatures summarized in Figure 4 yields an average denitrification rate of  $155 \text{ mg m}^{-2} \text{ d}^{-1}$  from March to June 2004 and  $4 \text{ mg m}^{-2} \text{ d}^{-1}$  from July to October (Table 2). The annual denitrification amounted to  $39 \text{ g m}^{-2} \text{ yr}^{-1}$ , if the spring denitrification rate was applied to all months with high  $\text{NO}_3^-$  and oxygen concentrations (November–June). It matched well with the denitrification rate of  $42 \text{ g m}^{-2} \text{ yr}^{-1}$  determined as the difference between the 2.5-yr N removal and N accumulation in the sediment. Hence, coupled nitrification–denitrification in the sediment, which does not imprint on the  $\delta^{15}\text{N}_{\text{NO}_3}$  in the water column and which was not assessed with the isotope approach, seemed to be of minor importance.

Nitrification determined from the isotope mass balance was on average  $91 \text{ mg m}^{-2} \text{ d}^{-1}$  and mostly fueled by  $\text{NH}_4^+$  released from the sediment (Table 2). In spring, DON mineralization may have contributed  $49 \text{ mg m}^{-2} \text{ d}^{-1}$  to the  $\text{NH}_4^+$  production. Benthic  $\text{NH}_4^+$  release calculated as the difference between nitrification and  $\text{NH}_4^+$  supply from DON ammonification or allochthonous  $\text{NH}_4^+$  ranged from  $43$  to  $177 \text{ mg m}^{-2} \text{ d}^{-1}$ . The benthic  $\text{NH}_4^+$  release from June to August ( $131 \text{ mg m}^{-2} \text{ d}^{-1}$ ) agreed remarkably well with the turbulent

diffusive  $\text{NH}_4^+$  flux ( $130 \text{ mg m}^{-2} \text{ d}^{-1}$ ) independently calculated from  $\text{NH}_4^+$  concentration gradients in the water column of the wetland (Figure 2c).

**Denitrification versus Nitrogen Recycling.** The average 2.5-yr denitrification rate ( $42 \text{ g m}^{-2} \text{ yr}^{-1}$ ) was at the upper level of the rates reported for lake, river and estuary sediments (21), but similar to values observed in wetlands (28). Benthic denitrification rates in Wetland Boden increased with increasing  $\text{NO}_3^-$  concentration in the water column as the laboratory experiments showed (Figure 3). This indicates that benthic denitrification is controlled by the diffusive  $\text{NO}_3^-$  supply to the sediment which increases in proportion to the  $\text{NO}_3^-$  concentration in the sediment overlying water. In accordance with that, high denitrification rates in spring 2004 were linked to high external  $\text{NO}_3^-$  loading and intense nitrification in the water column (Table 2). In contrast, low denitrification rates were observed in summer when  $\text{NO}_3^-$  concentrations declined because external  $\text{NO}_3^-$  load decreased and biomass production outcompeted denitrification.

The intense duckweed production provided only short-term N sink and fed internal N recycling. The N content of the settling matter mainly composed of duckweed ( $12.2 \text{ mg g}^{-1} \text{ dwt}$ ) was significantly higher than the N content of the sediment ( $2.5 \text{ mg g}^{-1} \text{ dwt}$ ). This indicates that at least 80% of the settled N was released during benthic mineralization. Due to little supportive tissues and low C:N ratios, floating leaved duckweed and phytoplankton are easily degradable and have been shown to lose up to 75% of their initial biomass within 10 weeks of decomposition (29, 30). Benthic release rates and the N content of the remaining organic sediment in Wetland Boden were similar to those of plankton-dominated lakes (31–34). In contrast, the typical N content reported for peatland soils (35, 36) was approximately 5–10 times higher than in Wetland Boden, suggesting that rooting macrophytes lead to a better N conservation and long-term storage than duckweed and phytoplankton.

As the burial of N in the sediment was of minor importance for long-term N removal, denitrification governed N-removal efficiency in Wetland Boden. Two contrasting situations developed (Figure 4), which are typical for wetlands with a variable water discharge and N-loading regime: TN removal was high when the  $\text{NH}_4^+$  released from the sediment was nitrified in the oxic water and subsequently denitrified in the anoxic sediment. However, N removal was close to zero during stagnant periods, when  $\text{NH}_4^+$  accumulated in the anoxic water layer and photoautotrophic plants intensively assimilated N.

To improve the N-removal efficiency of wetlands for treating nonpoint source pollution it is essential to minimize internal N recycling and to maximize denitrification losses. As benthic denitrification depends on high nitrate concentrations (Figure 3), the water layer above the anoxic sediments has to remain oxic to guarantee complete nitrification of  $\text{NH}_4^+$ . Keeping the wetland shallow can prevent stratification. Harvesting of floating leaved plants stimulates phytoplankton growth and oxygen production and further adds to the removal of the recyclable biomass N pool. However, complete biomass and sediment removal should be avoided to provide sufficient organic carbon substrate for denitrification. Our study has shown that isotopic analysis of the relevant N pools can help to disentangle complex and variable nitrogen elimination pathways and help to design management strategies for such artificial wetlands.

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## Supporting Information Available

A map of the study site and the correlation between surface specific N input and removal of Wetland Boden and other lakes and wetlands. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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