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Plant Responses to Nitrogen Fertilization Differ between Post-Mined and Original Peatlands

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Abstract Nitrogen addition experiments were conducted in three plant communities in a post-mined peatland and on the surrounding original *Sphagnum* bog in northern Japan to compare the effects of nitrogenous fertilization among the plant communities. Gradients of added nitrogen were prepared by adding nitrogen to 1 m × 1 m plots at rates of 0–36 g/m^2/yr. These different levels of nitrogen addition were applied to four sites representing a vegetation development chronosequence: bare ground (BG), a *Rhynchospora alba* sedgeland (RA), a *Moliniopsis japonica* grassland (MJ) and the original *Sphagnum* bog (SS). Vegetation was monitored in each plot for three years, and the groundwater level and nitrogen concentration in peat-pore water were monitored in each plot. The nitrogen concentration in peat-pore water increased with increasing nitrogen addition on BG, but was constantly low at the vegetated sites, suggesting the occurrence of nutrient uptake by plants. Species richness decreased as a result of nitrogen addition at the SS site, where plant cover was high. The aboveground biomass of the two common grasses *M. japonica* and *Phragmites communis* was not influenced by nitrogen addition in the post-mined peatland. Grasses and sedges, which produce large underground organs, showed increased biomass as a result of nitrogen addition in the SS plot whereas *Sphagnum* declined. Additionally, forbs declined in the SS plot with increasing nitrogen, most likely due to competition with the increased populations of grasses. Therefore, nitrogen enrichment can promote the dominance grasses, leading to a decrease in mosses and forbs.

Keywords Growth form · Revegetation · Nitrogen addition · Peat mining · Restoration · Peatland · *Sphagnum* bog · Ombrotrophic bog

Plant nomenclature:

Ohwi (1975), Tagawa (1983), Iwatsuki and Mizutani (1972)

Introduction

Sphagnum-dominated peatlands are especially important ecosystems for conserving rare species that inhabit specific environments (Keddy 2000). Moreover, because peatland ecosystems function as carbon sinks, in addition to protecting biological values such as biodiversity and the occurrence of rare species, the potential effects of global change on wetlands might be of critical importance (Limpens et al. 2011). Globally, the area of peatlands has been drastically reduced by human activities such as peat mining. As a response to the present-day rarity of peatland vegetation, the conservation and restoration of peatlands has become a priority. Peat mining produces harsh hydrological and physical conditions for the most dominant genera of peat-forming plants, especially *Sphagnum* mosses, resulting in reduced peat growth (Kuhry and Bitt 1996; Price and Whitehead 2001). Inadequate spontaneous revegetation at mined sites may inhibit the restoration of peatland functions. The mechanisms underlying reduced peat accumulation are uncertain, necessitating experiments to facilitate active peat growth.

Bogs are nutrient-poor habitats that provide safe sites for weakly competitive and slowly growing species (Øien 2004), and are thus sensitive to nutrient enrichment (Van Duren and Pegtel 2000). In grassland ecosystems, species richness is reported to be inversely associated with nutrient status (Dupre et al. 2010; Stevens et al. 2004); in other words, their richness declines with increasing nutrient load. To our knowledge, such relationships have not been found in wetlands, although the alteration of nitrogen availability appears to degrade their species composition and community development (Bedford et al. 1999). When nitrogen availability changes, competitive balances between species change with it, accelerating subsequent species turnover (Limpens et al. 2003; Schrijver et al. 2011). With increasing nitrogen deposition, nitrogen-demanding species, such as the grass *Molinia caerulea* and the shrub *Betula pubescens*, invade bogs, which is accompanied by a decline in ombrotrophic species (Aerts et al. 1992; Tomassen et al. 2004a). The increased

nitrogen levels derived from peat decomposition alter the composition of plant growth forms in peat bogs; shrubs expand at the detriment of graminoid species, for example (Kool and Heijmans 2009). Information on growth forms is used to identify the role of individual plant species in ecosystem functioning and the responses of these species to environmental changes such as nitrogen deposition. Fertilization experiments are useful for understanding the impacts of nitrogen on plant communities and have been undertaken in boreal wetlands, where substantial amounts of peat are accumulated. Such experiments have been conducted especially in Europe and North America (Limpens et al. 2011). In Japanese wetlands, nitrogen addition experiments are rare. It is therefore difficult to extrapolate to these valuable ecosystems.

Commercial *Sphagnum* peat mining, which has been widespread in boreal regions, completely removes standing vegetation (Zoltai and Vitt 1995). At the Sarobetsu Mire in northern Hokkaido, peat mining occurred annually from 1970 until 2003. Succession from bare ground to a grassy marshland has been detected in one post-mined peatland (Nishimura et al. 2009), although the original vegetation has not recovered more than 30 years after abandonment because of altered water level and chemistry after peat mining. Whenever restoration of an original community is planned in a damaged peatland, an understanding of the effects of nitrogen on vegetation dynamics is required because peat mining increases nitrogen levels in both ground water and peat (Wind-Mulder and Vitt 2000) and because nutrient availability can generally influence the pace and direction of vegetation recovery after a disturbance (Wilson and Tilman 2002; Baer et al. 2004). Furthermore, the responses of plants to increased nitrogen differ among growth forms (i.e. forbs, grasses, shrubs and mosses) in northern peatlands (Dorrepaal et al. 2005). In this study, we aimed to test the impact of increased nitrogen levels on vegetation restoration after peat mining. We therefore conducted a nitrogen addition experiment in pre- and post-mined peatland sites, and for three years monitored nitrogen availability in peat-pore water and the structure of the vegetation in terms of dominant species and growth forms.

Materials and methods

Study area and experimental design

The field experiment was conducted in the Sarobetsu Mire, northern Hokkaido, Japan (45°06′N, 141°42′E, 7 m a.s.l.). Currently, the Sarobetsu Mire is covered by a 2,773 ha *Sphagnum* peat bog, which is approximately 6 m deep (Hotes et al. 2004). From 2005 until 2007, when the surveys were conducted, the mean annual temperature in the town of Toyotomi, 6 km east of the study site, was 6.5°C, with monthly averages ranging from −12.6°C in February 2005 to 25.4°C in August 2006 (Japan Meteorological Agency 2008). Annual precipitation averaged 955 mm. The snow-free period usually extends from April to November. Over the past 30 years, the maximum snow depth was 114 cm.

Sphagnum peat had been excavated for commercial use at a rate of 3–22 ha/year to a depth of more than 3 m between 1970 and 2003. To compare the effects of nitrogen addition among plant communities, we selected four sites representing a chronosequence. A chronosequence analysis (Nishimura et al. 2009) has indicated that the three communities at the post-mined sites changed from bare ground (BG) to a *Moliniopsis japonica* grassland (MJ) via an intermediate *Rhynchospora alba* sedgeland (RA) stage. Three sites were selected to represent post-mined sites (Nishimura et al. 2009): BG, mined in 1982 with low plant cover and richness; RA sedgeland, mined in 1975 and with greater than 80 % cover; and MJ grassland, mined in 1970 and with 70–80 % cover. An additional site was established in the surrounding unmined bog (SS) with a predominance of *Sphagnum* spp. (primarily *Sphagnum papillosum* and *S. magellanicum*). At each site, 25 1 m × 1 m plots were set up in the early spring of 2005 at 3–5 m intervals within a 0.25 ha area. Five treatments were applied, differing in nitrogen concentrations and producing nitrogen loads of 0.0,

4.5, 9.0, 18.0 and 36.0 g $N/m^2/yr$ via nitrogen addition. Because the Sarobetsu Mire is surrounded by agricultural fields, nutrient levels are high in rivers, lakes and the downstream basin near the bog under study (Tachibana et al. 1996). Nitrogen was applied as granular urea, $CO(NH_2)_2$, an aldehyde condensation compound that slowly and continuously releases ammonium (NH_4^+) upon hydrolysis. Granular urea was supplied seven times during the snow-free period between April 2005 and October 2006 because plants, *Sphagnum* in particular, utilize NH_4^+ more than they utilize NO_3^- in nutrient-poor, acidic and wet habitats (Jauhiainen et al. 1998; Larcher 2003). To eliminate plot edge effects, urea was also sprayed 20 cm beyond the plots. Each concentration treatment consisted of five replicates.

Measurements and sampling

In early September of each year, the plant cover was visually estimated for each species in each plot. For this purpose, each plot was divided into 25 cells using a 1 m × 1 m frame. The nomenclature follows Ohwi (1975) for vascular plants, Tagawa (1983) for ferns, and Iwatsuki and Mizutani (1972) for mosses. In the summer of 2006, shoots of the two dominant grasses, *Phragmites communis* and *M. japonica*, were counted. Shoots of *P. communis* were counted in each 1 m × 1 m plot, and shoots of *M. japonica* were counted within a 20 cm × 20 cm subplot established in each plot. In 2007 shoots were counted in each plot for *P. communis* and within 40 cm × 40 cm subplots in each plot for *M. japonica* and *Carex middendorffii*. *S. capitulum* density was measured as a surrogate for vitality (Breeuwer et al. 2008). We performed this density measurement by counting the number of capitula in a 20 cm^2 circle placed randomly within each 1 m × 1 m plot in 2006 and 2007. In September 2007 vascular plant aboveground biomass was harvested in a 40 cm × 40 cm subplot except for *P. communis*, which was harvested in the 1 m × 1 m plots. *Sphagnum* biomass was sampled using 100 cm^3 cores (20 cm^2 in surface area and 5 cm in depth) from three locations in

each plot. Dead *Sphagnum* tissue, which was brown in colour, was excluded from the biomass samples (Güsewell et al. 2002). The samples of each species were weighed after drying at 65°C for 72 h. The vegetation was classified into four growth forms: shrubs, forbs, grasses (including sedges) and mosses (mostly *Sphagnum*; refer to Chapin et al. 1996). The nitrogen content in leaves was measured for the four dominant species (*M. japonica*, *P. communis*, *R. alba* and *C. middendorffii*) and *Sphagnum* spp. The plants were harvested with shears at the ground surface at the three vegetated sites, RA, MJ and SS, if the amount of available biomass was sufficient for harvesting. The nitrogen content of dry shoots (weighing 10–20 mg) was measured with an NC analyser (NC-900, Sumika Chemical Analysis Service, Ltd., Tokyo, Japan). The calibration curve was obtained using acetanilide.

The water level was measured using four PVC pipes (1 m in length and 4 cm in diameter) inserted 85 cm below the peat surface at each site. Holes 5 mm in diameter were made in the pipes at 10 cm intervals by drilling, and one end was sealed before use. The water level was measured during the snow-free period between August 2005 and October 2007. In this study, a positive value of the water level indicates that groundwater appears above the ground surface.

Groundwater chemistry was measured before the experiment using 100 ml of groundwater collected from inside previously established PVC pipes in June, August and November 2003. Electrical conductivity (EC) and pH were measured using an EC and pH meter, respectively (MP120 and MP125, Mettler Toledo, Tokyo), in the laboratory within a day or two after the samples were brought to the laboratory. The sampled water was then filtered through a 0.2 μm cellulose acetate membrane filter (Advantec Toyo Co., Tokyo) with a filter bell to reduce microbial activity. The water samples were frozen until analysis. NH_4^+ and NO_3^- concentrations were analysed using an ion chromatographer (DX-120, Japan Dionex, Tokyo). Total nitrogen (TN) was measured by the Griess-Ilosvay method (Keeney and Nelson 1982). A sample of 100 ml groundwater was collected from the inside of each PVC pipe in April 2005 before nitrogen addition. In October 2006 and May,

August and October 2007, 20 ml of peat-pore water was extracted from the peat layer to a depth of 20 cm from the surface using a porous tube (DIK-8392, Daiki Rika, Shiga). This water sample was handled with the same procedures as used for groundwater. Concentrations of NH_4^+, NO_3^- and NO_2^- in the water were measured with an automatic water analyser (QuAATro TNTP Multi, Bran Luebbe Co., Tokyo).

Data handling and statistical analysis

Annual changes in vegetation composition were evaluated by percentage dissimilarity (PD):

$$\mathrm{PD}=\left\{1-\left(\frac{2\times\sum_{i=1}^{n}\min(x_i,y_i)}{\sum_{i=1}^{n}(x_i+y_i)}\right)\right\}\times 100,$$

where n is the total number of species observed in the two years (x and y), and x_i and y_i are the percentage cover of species i in the two years. Here, $\min(x_i, y_i)$ is the minimum value of the percentage cover of species i in the two years (Daha E and Hadač 1941). Shannon's diversity index (H') was calculated in each plot as follows:

$$H'=-\sum_{i=1}^{n} p_i \ln p_i ,$$

where p_i is the proportion of the cover of each species to the total cover.

The groundwater chemistry in 2003 was compared among vegetation types by performing a one-way repeated-measures ANOVA with a Tukey *post hoc* test. The total nitrogen (TN) concentration, the ratio between the NH_4^+ and NO_3^- concentrations in pore water, the plant cover, the species richness, the diversity, the shoot densities and the capitulum density of *Sphagnum* were compared among nitrogen addition rates across experimental periods using a one-way repeated-measures ANOVA to perform a time series analysis (i.e. using the year or month as repeated-measures data). The TN concentration and the ratio between the NH_4^+ and NO_3^-

concentrations in pore water were analysed with a linear mixed-effects model with the amount of nitrogen addition as a continuous variable to conduct a time series analysis. Vegetation dissimilarity between 2005 and 2007, biomass of dominant species and growth forms, and the nitrogen standing stock (g/m^2) of dominant species were compared among the nitrogen addition rates by a one-way ANOVA and a Tukey *post hoc* test. The biomass data were log-transformed [log (biomass + 0.001)]. All statistical analyses were conducted in R 2.12.1 (R Core Team 2010). The one-way repeated-measures ANOVA was analysed using the 'car' package (v. 2.0–11), and the linear mixed-effects model was analysed with the 'nlme' package (v. 3.1–98).

Results

Water chemistry in peat

Prior to establishing the experimental plots, the chemical properties TN, NH_4^+, pH and EC of groundwater differed among the four community types whereas no differences among community types were found for NO_3^- (Table **1**). TN and NH_4^+ were lower in the original peatland (SS) than at the BG and MJ sites, but their values at the SS site did not differ significantly from their values at the RA site. Among the post-mined sites (BG, RA and MJ), NH_4^+ concentrations as well as the pH and EC were significantly higher on BG, and TN concentrations at the BG and MJ sites were significantly higher than those at the RA site. These results indicate that nitrogen availability in groundwater generally tended to be higher in the mined peatlands than on the unmined original bog. Moreover, the groundwater chemistry at the RA sites was similar to that at the unmined (SS) site.

Precipitation was primarily supplied by rainfall between May and October over the three years (Fig. **1**). The water level in RA was relatively stable and high, and was more than 5 cm higher than that in any other site throughout the snow-free period. The patterns of water level fluctuation were

similar in MJ and SS. BG showed the lowest water level and responded to rainfall more rapidly than any other vegetated site. Nitrogen addition affected the TN concentration at the BG and MJ sites (Fig. **2**). The TN concentration in BG and MJ plots significantly increased with nitrogen addition (BG: *d.f.*=4, F=10.04, P=0.002; MJ: *d.f.*=4, F=3.80, P=0.040) and varied over time (BG: *d.f.*=3, F=9.38, P<0.001; PM: *d.f.*=3, F=3.42, P=0.030). Additionally, TN was higher in the BG plot than at the other sites in all seasons. The TN concentration at the RA and SS sites differed significantly among months (RA: *d.f.*=3, F=7.35, P<0.001; SS: *d.f.*=3, F=12.72, P<0.001). The ratio between the NH_4^+ and NO_3^- concentrations was not significantly affected by nitrogen addition at any site. In the RA plot, this ratio fluctuated significantly among months, as shown by the repeated-measures data (*d.f.*=3, F=3.05, P<0.001).

Responses of plant communities to nitrogen addition

Plant cover, species richness and diversity were lowest on BG over the three study years (Fig. **3**) because only three monocotyledonous species, *R. alba*, *M. japonica* and *C. middendorffii*, were recorded; moreover, *R. alba* was dominant. Because the plant cover was always lower than 2 % on BG, nutrient uptake by plants can be excluded from this site; therefore, the effects of nitrogen addition on pore water chemistry can be interpreted as true effects. Species richness and diversity had a tendency to decrease in response to high nitrogen fertilization in the second experimental year, but recovered in the following year. Only diversity showed a statistically significant annual change (*d.f.*=2, F=4.01, P=0.03).

In the RA plot, the plant cover ranged from 40 % to 80 %, including eight forbs, three grasses, four sedges and five mosses. In the RA control plots, the most dominant species was *R. alba*, with a total cover of 46–61 %; the total cover of *C. middendorffii*, *S. papillosum* and *M. japonica* was 5–16 % in each case. The plant cover varied significantly over time (*d.f.*=2, F=13.20, P<0.001), and

these patterns of variation differed, with a significant interaction between year and nitrogen addition. Nitrogen addition did not change species richness or diversity (*d.f.*=4, *F*=0.11, *P*=0.98; *d.f.*=4, *F*=1.05, *P*=0.40, respectively; Fig. **3**).

In the MJ control plots, *M. japonica* showed a total cover of 37–55 % and was most dominant. The composition of growth forms was similar to that in the RA plot (i.e. nine forbs, three grasses, four sedges, five shrubs and three mosses). In the MJ plot without nitrogen addition, *C. middendorffii* and *P. communis* were dominant, with more than 10 % cover, and *R. alba*, *S. papillosum* and *Myrica gale* were also present. *M. gale* is a low shrub and showed a total cover of approximately 10 %. Among the forbs, *Lobelia sessilifolia* and *Hosta rectifolia* were the most frequent, showing a low total cover of 2–3 %. Nitrogen addition did not influence the plot cover (*d.f.*=4, *F*=0.88, *P*=0.49), species richness (*d.f.*=4, *F*=0.88, *P*=0.50) or diversity (*d.f.*=4, *F*=0.11, *P*=0.98; Fig. **3**).

In the original bog (SS), there were 12 species of forbs, one grass, one sedge, six shrubs and five mosses. In the control plots, *S. papillosum* was the most common, with a cover of more than 80 %. *S. magellanicum* was also well established, with a cover of 8–14 %. Shrub richness in the SS plot was the highest of the four sites and was represented by *Empetrum nigrum*, *M. gale* and *Vaccinium oxycoccus*. Among vascular plants, *C. middendorffii* had the highest cover, ranging from 20–36 % in the control, whereas the cover of the other vascular plants was less than 10 %. It is probable that mosses took up more nutrients than vascular plants in the SS plot because of the dominance of *Sphagnum*. In contrast, the relative role of mosses in nutrient uptake was most likely less marked in the RA and MJ plots. The plant cover was significantly affected by nitrogen addition (*d.f.*=4, *F*=22.59, *P*<0.001) and the year (repeated-measures analysis, *d.f.*=2, *F*=78.06, *P*<0.001), with a significant interaction between the two factors (*d.f.*=8, *F*=15.28, *P*<0.001; Fig. **3**), indicating that the response of the plant cover over the experimental period differed among the nitrogen addition treatments. Species richness in the SS plot differed significantly as a result of nitrogen

addition (*d.f.*=4, *F*=4.01, *P*=0.015) and in the second year (*d.f.*=2, *F*=17.14, *P*<0.001). Similarly, diversity at the SS site responded significantly to nitrogen addition (*d.f.*=4, *F*=3.00, *P*=0.043) and fluctuated annually (*d.f.*=2, *F*=6.79, *P*<0.001; Fig. **3**). Overall, the SS site would be more susceptible to a change in nitrogen conditions than the vegetated sites. Accordingly, the species composition at the SS site would tend to change easily.

Temporal changes in vegetation structure, expressed by dissimilarity, showed significant differences at the RA site (*d.f.*=4, *F*=5.54, *P*=0.004) as a result of nitrogen addition (Fig. **4**). The dissimilarity increased with increasing nitrogen addition, particularly in the RA plot, implying that the vegetation structure was altered more substantially by nitrogen addition at this site.

Responses of biomass to nitrogen addition

The analysis of aboveground biomass harvested in the third year (Fig. **5**) showed that nitrogen addition did not influence the biomass of the two grasses *P. communis* and *M. japonica* in any of the plots. With increasing nitrogen addition, *R. alba* tended to decrease in RA and MJ plots, but these results were not statistically significant (RA: *d.f.*=4, *F*=1.50, *P*=0.24; MJ: *d.f.*=4, *F*=0.19, *P*=0.94). *C. middendorffii* showed increased biomass in the SS plot with increasing nitrogen addition (*d.f.*=4, *F*=3.22, *P*=0.03). This species tended to show increased biomass in the RA and MJ plots at the nitrogen addition rate of 36 g/m^2/yr, although the results were not statistically significant (RA: *d.f.*=4, *F*=0.66, *P*=0.63; MJ: *d.f.*=4, *F*=0.25, *P*=0.91). At the post-mined sites, nitrogen addition did not influence the biomass of any of the dominant species.

The responses of the biomass of each growth form to nitrogen addition differed among the sites (Fig. **6**). In particular, nitrogen addition increased grass biomass (*d.f.*=4, *F*=3.65, *P*=0.02) in the SS plot, but completely eliminated mosses (*d.f.*=4, *F*=42.5, *P*<0.001). The addition of 36 g N/m^2/yr to the SS plot decreased the biomass of mosses to near zero. Nitrogen addition caused the biomass of

forbs to decrease in the SS plot (*d.f.*=4, *F*=3.76, *P*=0.02) and tended to cause the biomass of forbs to decrease in the MJ plot (not significantly; *d.f.*=4, *F*=1.95, *P*=0.14) whereas no effect of nitrogen addition on forb biomass was found in the RA plot (*d.f.*=4, *F*=0.85, *P*=0.51). No statistically significant effect of nitrogen addition on shrub biomass was found in the RA or SS plots (RA: *d.f.*=4, *F*=1.06, *P*=0.40; SS: *d.f.*=4, *F*=0.85, *P*=0.51). In the MJ plot, the only effect of nitrogen addition on shrub biomass was increased biomass at the addition rate of 18 $g/m^2/yr$ (*d.f.*=4, *F*=2.85, *P*=0.05).

Capitulum density of *Sphagnum* increased with increasing nitrogen up to 18 $g/m^2/yr$ at all of the sites from 2006 to 2007, but decreased at the nitrogen addition rate of 36 $g/m^2/yr$ in the SS plot (Table **2**), showing that vitality decreased as a result of excessive nitrogen addition. *Myrica japonica* showed the highest shoot density in the RA plot except at the nitrogen addition rate of 9.0 $g/m^2/yr$ and showed a low shoot density in the MJ plot, indicating that this species developed well in the transitional *R. alba* grassland and then declined. The shoot density of *P. communis* in the RA plot was not changed by nitrogen addition and decreased slightly in the MJ plot with increasing time, indicating that nitrogen addition had the least influence on *P. communis*. Nitrogen addition tended to increase the shoot density of *C. middendorfii* in 2007.

Nitrogen concentration in plants

The nitrogen standing stock in *M. japonica* did not increase with increasing nitrogen addition at any of the three mined sites (Fig. **7**) whereas *P. communis*, which was not established in the SS plot, tended to show an increase in nitrogen standing stock as a result of nitrogen addition in the MJ plot, although this increase was not significant. Similarly, *C. middendorffii*, which was well established in the SS plot, tended to show an increase in nitrogen standing stock with increasing nitrogen addition in the SS plot, although this increase was not significant. *P. communis* was the largest grass, and *C. middendorffii* was the largest sedge. In total, therefore, large perennial herbs did not respond

well to nitrogen addition in the sparsely vegetated RA site, but showed increased leaf nitrogen concentrations at the densely vegetated MJ and SS sites. The nitrogen standing stock in *Sphagnum* decreased significantly with nitrogen addition in the MJ and SS plots ($d.f.$=4, F=9.80, P<0.001).

Discussion

Community responses to nitrogen addition

One of the objectives of ecological restoration is to accelerate ecosystem recovery, as evaluated in terms of community structure and diversity. After peat mining, the spontaneous recovery of a *Sphagnum* bog requires more than three decades and occasionally fails (Nishimura et al. 2009). Nitrogen addition did not influence the plant communities at the post-mined sites but decreased the plant cover, species richness and diversity at the unmined site. The most dominant vascular plants were large grasses and sedges. The decrease in species richness resulted primarily from a decrease in forbs, particularly at the SS site. Nitrogen fertilization enhances shoot competition via height growth rather than via root competition in peatlands (Limpens et al. 2003; Güsewell 2005; Kool and Heijmans 2009). With increasing nitrogen in the Sarobetsu Mire, grasses appeared to suppress the growth of forbs through shoot competition.

In this study site, the water level differed drastically between the vegetation types. The water level remained higher at the RA site year round than at the other mined sites. As a result, *Sphagnum* mosses were able to recolonize the site after the cessation of peat mining (Nishimura et al. 2009). Peat mining activities cause a lowering of the water level in many peatlands, resulting in harsh physical and hydrological conditions in the peat that limit successful recolonization by *Sphagnum* mosses (Price and Whitehead 2001). Thus, approaches involving water management may be important for improving the hydrological conditions within the peat substrate (Ketcheson and Price

2011).

Relationships between growth form and nitrogen addition

Grass biomass increased as a result of nitrogen addition in the SS plot whereas mosses and forbs decreased. Nutrient requirements differ among growth forms (i.e. forbs, grasses and shrubs) and between habitats (i.e. bogs and fens; Bowman et al. 1995). Two shrubs, *Vaccinium myrtillus* and *V. vitis-idaea*, which are dominant in bogs, show optimal growth if moderate nitrogen is supplied and decreased growth if excess nitrogen is supplied (Makipaa 1999). Moreover, nutrient addition greatly increases aboveground biomass by stimulating plant productivity and by shifting species composition from slow-growing species to productive species such as dwarf shrubs like *Betula nana* (Mack et al. 2004). Accordingly, grasses and shrubs increased as *Sphagnum* decreased in the SS plot. By contrast, although shrub biomass in the MJ plot showed a partial increase at the nitrogen addition rate of 18 $g/m^2/yr$, but at no other nitrogen addition rate, the biomass of grasses and shrubs did not change in the post-mined peatlands (i.e. the RA and MJ sites). Because less *Sphagnum* develops in post-mined peatlands, other stressors restrict the growth of grasses and shrubs (Koyama and Tsuyuzaki 2010).

In moss biomass, which consisted primarily of *Sphagnum*, excessive nitrogen addition caused withering. Excess ammonium ions are toxic for *Sphagnum* (Limpens and Berendse 2003). In oligotrophic bogs, *Sphagnum* growing above the surface of the peat directly absorbs nutrients supplied from precipitation before vascular plant roots below the surface can capture nutrients (Lamers et al. 2000). The *Sphagnum* layer, which functions as a nutrient filter, was destroyed by nitrogen addition. Therefore, this change may stimulate the growth of vascular plants with vertically elongated roots. *Sphagnum* is then further depressed by shading from the overstory of vascular plants as increasing nitrogen addition promotes their growth (Lamers et al. 2000; Berendse et al.

2001), suggesting that nitrogen addition indirectly restricts the growth of *Sphagnum*.

Responses of vascular plants to nitrogen addition to peat

Bare ground is subject to drought, strong wind and ground surface instability, all of which restrict seedling establishment (Eckstein and Donath 2005; Koyama and Tsuyuzaki 2010). Early recolonization of mined peatlands can reduce these stresses. Solutes in groundwater are transported upward to the bare ground by capillary action in peat and get concentrated by evaporation (Cobbaert et al. 2004). In addition, available nitrogen is enriched relative to the original bog in post-mined peatlands by oxidation under aerobic conditions (Andersen et al. 2006). In a post-mined peatland, the ammonium concentration becomes high due to the removal of *Sphagnum*, which requires ammonium nitrogen rather than nitric nitrogen (Jauhiainen et al. 1998; Tomassen et al. 2004b). Nitrogen concentrations were lower at the vegetated sites, particularly in the SS plot, than on BG, indicating that the plants absorbed nitrogenous ions from peat-pore water.

Temporal vegetation changes in the RA plot can be assumed to be caused by the reduction of *R. alba* as the dominant species. The aboveground biomass of *R. alba* tended to decrease with nitrogen addition in the RA plot, but turned out to be non-significant. *R. alba* is an early colonizer after peat mining (Nishimura et al. 2009) and is a weak competitor for nutrients obtained from peat (Ohlson and Malmer 1990). Nitrogen addition substantially increases *R. alba* biomass if interspecific competition is excluded (Heijmans et al. 2002; Egawa et al. 2009). The biomass of *R. alba* tended to decrease with nitrogen addition at the post-mined sites. In particular, such decreases were observed in the RA plot, where *R. alba* was dominant. Moreover, increased nitrogen deposition potentially increases plant phosphorus uptake in a species-specific way due to changes in the N:P ratio (Fujita et al. 2010), which may cause another nutrient, such as phosphorus, to become limiting for *R. alba*. Therefore, *R. alba* declines if the nitrogen concentration is high and interspecific

competition is intense. These results also mean that the successional change from early to late colonizers is promoted by nitrogen enrichment.

Most species of the genus *Molinia* (*Moliniopsis*, *sensu lato*) are nitrophilous grasses and can invade ombrotrophic bogs after nitrogen deposition occurs in association with a decline in bog species (Limpens et al. 2003). Nitrogen addition increased the aboveground biomass and nitrogen content of *M. japonica* in the SS plot except in the case of the highest concentration of nitrogen, suggesting that nitrogen limited the growth of *M. japonica* on the *Sphagnum* bog if this nutrient was not in excess. A decline in *Sphagnum* in the SS plot should be advantageous for the growth of *M. japonica* because nitrogen uptake by *Sphagnum* declines. *Moliniopsis* regenerates well from seedlings on turf with high nitrogen levels (Tomassen et al. 2003), and *M. japonica* develops a short-term persistent seedbank (Egawa et al. 2009). Therefore, *M. japonica* increased its shoot density by seedling regeneration after nitrogen addition, but did not change its biomass.

P. communis was established least densely on the original bog at the Sarobetsu Mire whereas this grass is tallest in the post-mined peatland (Nishimura et al. 2009). In wetlands in Illinois, USA, nitrogen addition enhances the growth of *P. communis*, which is an invasive grass, more than the native grass *Spartina pectinata* (Rickey and Anderson 2004). An invasive grass, *Phalaris arundinacea*, grows rapidly when resource limitation is released by nitrate addition and then suppresses native species (Green and Galatowitsch 2002). *P. communis* does not develop a persistent seedbank in various wetlands, including the Sarobetsu Mire (Egawa et al. 2009), and reproduces primarily by vegetative reproduction under various nutrient conditions (Bart and Hartman 2002; Alvarez et al. 2005). The highest leaf nitrogen concentration of *P. communis* was stable and high at various rates of nitrogen addition to peat, most likely because long rhizomes and stolons utilize nutrients in broader areas.

In conclusion, the mechanisms of altering nitrogen concentration in peat should be clarified before restoring *Sphagnum* bogs. High nitrogen availability harmed original bog species, such as

Sphagnum mosses, and promoted the immigration of *P. communis*, which did not occur on the original bog. Nitrogen accumulation in peat should therefore be avoided not only to conserve the original bog, but also to promote the revegetation of the post-mined peatland, although the effects and mechanisms by which nitrogen addition produces vegetation changes differ between pre-and post-mined peatlands.

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References

Aerts R, Wallen B, Malmer N (1992) Growth-limiting nutrients in *Sphagnum*-dominated bogs subject to low and high atmospheric nitrogen supply. *J Ecol* 80:131–140

Alvarez M, Tron GF, Mauchamp A (2005) Sexual versus asexual colonization by *Phragmites australis*: 25-year reed dynamics in a Mediterranean marsh, southern France. *Wetlands* 25:639–647

Andersen R, Francez AJ, Rochefort L (2006) The physicochemical and microbiological status of a restored bog in Quebec: Identification of relevant criteria to monitor success. *Soil Biol Biochem* 38:1375–1387

Baer SG, Blair JM, Collins SL, Knapp AK (2004) Plant community responses to resource availability and heterogeneity during restoration. *Oecologia* 139:617–629

Bart D, Hartman JM (2002) Environmental constraints on early establishment of *Phragmites australis* in salt marshes. *Wetlands* 22:201–213

Bedford BL, Walbridge MR, Aldous A (1999) Patterns in nutrient availability and plant diversity of temperate North American Wetlands. *Ecology* 80:2151–2169

Berendse F, van Breemen N, Rydin H, Buttler A, Heijmans M, Hoosbeek MR, Lee JA, Mitchell E, Saarinen T, Vasander H, Wallén B (2001) Raised atmospheric CO_2 levels and increased N deposition cause shifts in plant species composition and production in *Sphagnum* bogs. *Global Change Biol* 7:591–598

Bowman WD, Theodose TA, Fisk MC (1995) Physiological and production responses of plant growth forms to

increases in limiting resources in alpine tundra: implications for differential community response to environmental change. *Oecologia* 101:217–227

Breeuwer A, Heijmans MMPD, Robroek BJM, Berendse F (2008) The effect of temperature on growth and competition between *Sphagnum* species. *Oecologia* 156:155–167

Chapin III FS, Bret-Harte MS, Hobbie SE, Zhong H (1996) Plant functional types as predictors of transient responses of arctic vegetation to global change. *J Veg Sci* 7:347–358

Cobbaert D, Rochefort L, Price JS (2004) Experimental restoration of a fen plant community after peat mining. *Appl Veg Sci* 7:209–220

Daha E, Hadač E (1941) Strandgesellschaften der Insel Ostoy im Oslofjord. *Nytt Mag Naturvidensk* 82:251–312

Dorrepaal E, Cornelissen JHC, Aerts R, Wallen B and van Logtestijn RSP (2005) Are growth forms consistent predictors of leaf letter quality and decomposability across peatlands along a latitudeinal gradient? *J Ecol* 93:817–828

Dupre C, Stevens CJ, Ranke T, Bleeker A, Peppler-Lisbach C, Gowing DJG, Dise NB, Dorland E, Bobbink R, Diekmann M (2010) Changes in species richness and composition in European acidic grasslands over the past 70 years: the contribution of cumulative atmospheric nitrogen deposition. *Global Change Biol* 16:344–357

Eckstein RL, Donath TW (2005) Interactions between litter and water availability affect seedling emergence in four familial pairs of floodplain species. *J Ecol* 93:807–816

Egawa C, Koyama A, Tsuyuzaki S (2009) Relationships between the developments of seedbank, standing vegetation and litter in a post-mined peatland. *Pl Ecol* 203:217–228

Fujita Y, Robroek BJM, de Ruiter PC, Heil GW, Wassen MJ (2010) Increased N affects P uptake of eight grassland species: the role of root surface phosphatase activity. *Oikos* 119:1665–1673

Green EK, Galatowitsch SM (2002) Effects of *Phalaris arundinacea* and nitrate-N addition on the establishment of wetland plant communities. *J Appl Ecol* 39:134–144

Güsewell S, Koerselman W, Verhoeven JTA (2002) Time-dependent effects of fertilization on plant biomass in floating fens. *J Veg Sci* 13:705–718

Güsewell S (2005) High nitrogen: phosphorus ratios reduce nutrient retention and second-year growth of wetland sedges. *New Phytol* 166:537–550

Heijmans MM, Klees H, de Visser W, Berendse F (2002) Response of a *Sphagnum* bog plant community to elevated CO_2 and N supply. *Pl Ecol* 162:123–134

Hotes S, Poschlod P, Takahashi H, Grootjans AP, Adema E (2004) Effects of tephra deposition on mire vegetation: a

field experiment in Hokkaido, Japan. *J Ecol* 92:624–634

Iwatsuki Y, Mizutani M (1972) *Colored illustrations of bryophytes of Japan.* Hoikusya Publishing, Osaka

Jauhiainen J, Wallén B, Malmer N (1998) Potential NH_4^+ and NO_3^- uptake in seven *Sphagnum* species. *New Phytol* 138:287–293

Japan Meteorological Agency (2007) Available at: http://www.data.jma.go.jp/obd/stats/etrn/index.php

Keddy PA (2000) *Wetland ecology principles and conservation.* Cambridge University Press, Cambridge

Keeney DR, Nelson DW (1982) Methods of soil analysis. Part. 2. Chemical and microbiological properties, 2nd ed. In Page AL, Miller RH, Keeney DR (eds) *Nitrogen-inorganic forms*. American Society of Agronomy and Soil Science of America, Madison, pp 643–698

Ketcheson SJ, Price JS (2011) The impact of peatland restoration on the site hydrology of an abandoned block-cut bog. *Wetlands* 31:1263–1274

Koyama A, Tsuyuzaki S (2010) Effects of sedge and cottongrass tussocks on plant establishment patterns in a post-mined peatland, northern Japan. *Wetlands Ecol Managem* 18:135–148

Kool A, Heijmans MMPD (2009) Dwarf shrubs are stronger competitors than graminoid species at high nutrient supply in peat bogs. *Pl Ecol* 204:125–134

Kuhry P, Vitt DH (1996) Fossil carbon/nitrogen ratios as a measure of peat decomposition. *Ecology* 77:271–275

Lamers LPM, Bobbink R, Roelofs JGM (2000) Natural nitrogen filter fails in polluted raised bogs. *Global Change Biol* 6:583–586

Larcher W (2003) *Physiological plant ecology.* Ed. 4. Springer-Verlag, Berlin, Heidelberg, New York

Limpens J, Berendse F (2003) Growth reduction of *Sphagnum magellanicum* subjected to high nitrogen deposition: the role of amino acid nitrogen concentration. *Oecologia* 135:339–345

Limpens J, Berendse F, Klees H (2003) N deposition affects N availability in interstitial water, growth of *Sphagnum* and invasion of vascular plants in bog vegetation. *New Phytol* 157:339–347

Limpens J, Granath G, Gunnarsson U, Aerts R, Bayley S, Bragazza L, Bubier J, Buttler A, van den Berg LJL, Francez AJ, Gerdol R, Grosvernier P, Heijmans MMPD, Hoosbeek MR, Hotes S, Ilomets M, Leith I, Mitchell EAD, Moore T, Nilsson MB, Nordbakken JF, Rochefort L, Rydin H, Sheppard LJ, Thormann M, Wiedermann MM, Williams BL, Xu B (2011) Climatic modifiers of the response to nitrogen deposition in peat-forming *Sphagnum* mosses: a meta-analysis. *New Phytol* 191:496–507

Mack MC, Schuur EAG, Bret-Harte MS, Shaver GR, Chapin III FS (2004) Ecosystem carbon storage in arctic Tundra reduced by long-term nutrient fertilization. *Nature* 431:440–443

Makipaa R (1999) Response patterns of *Vaccinium myrtillus* and *V. vitis-idaea* along nutrient gradients in boreal forest. *J Veg Sci* 10:17–26

Nishimura A, Tsuyuzaki S, Haraguchi A (2009) A chronosequence approach fro detecting revegetation patterns after *Sphagnum*-peat mining, northern Japan. *Ecol Res* 24:237–246

Ohlson M, Malmer N (1990) Total nutrient accumulation and seasonal variation in resource allocation in the bog plant *Rhynchospora alba*. *Oikos* 58:100–108

Ohwi J (1975) *Flora of Japan* (revised edn). Shibundo, Tokyo

Øien DI (2004) Nutrient limitation in boreal rich-fen vegetation: A fertilization experiment. *Appl Veg Sci* 7:119–132

Price JS, Whitehead GS (2001) Developing hydrologic thresholds for *Sphagnum* recolonization on an abandoned cutover bog. *Wetlands* 21:32–40

R Core Team (2014) R: A Language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Available at http://www.R-project.org

Rickey M, Anderson RC (2004) Effects of nitrogen addition on the invasive grass *Phragmites australis* and a native competitor *Spartina pectinata*. *J Appl Ecol* 41:888–896

Schrijver AD, De Frenne P, Ampoorter E, Van Nevel L, Demey A, Wuyts K, Verheyen K (2011) Cumulative nitrogen input drives species loss in terrestrial ecosystems. *Global Ecol Biogeogr* 20:803–816

Stevens CJ, Dise NB, Mountford JO, Gowing DJ (2004) Impact of nitrogen deposition on the species richness of grasslands. *Science* 303:876–1879

Tachibana H, Hotta S, Minamide M, Saito H, Kawamura T (1996) Chemical characteristics of ground water and surface water in Sarobetsu Mire and its surrounding area. *J Water Environm* 19:910–921

Tagawa M (1983) *Colored illustrations of the Japanese pteridophyta*. Hoikusya Publishing, Osaka

Tomassen HM, Smolders AJP, Lamers LPM, Roelofs JGM (2003) Stimulated growth of *Betula pubescens* and *Molinia caerulea* on ombrotrophic bogs: role of high levels of atmospheric nitrogen deposition. *J Ecol* 91:357–370

Tomassen HBM, Smolders AJP, Limpens J, Lamers LPM, Roelofs JGM (2004a) Expansion of invasive species on ombrotrophic bog: desiccation or high N deposition? *J Appl Ecol* 41:139–150

Tomassen HBM, Smolders AJP, Lamers LPM, Roelofs JGM (2004b) Development of floating rafts after the rewetting of cut-over bogs: the importance of peat quality. *Biogeochemistry* 71:69–87

Van Duren IC, Pegtel DM (2000) Nutrient limitations in wet, drained and rewetted fen meadow: evaluation of methods and results. *Pl & Soil* 220:35–47

Wilson SD, Tilman D (2002) Quadratic variation in old-field species richness along gradients of disturbance and

nitrogen. *Ecology* 83:492–504

Wind-Mulder HL, Vitt DH (2000) Comparisons of water peat chemistries of a post-harvested and undisturbed peatland with relevance to restoration. *Wetlands* 20:616–628

Zoltai SC, Vitt DH (1995) Canadian wetlands: Environmental gradients and classification. *Vegetatio* 118:131–13

Fig. 1 Seasonal fluctuations in water level (mean ± SD, *n*=4) measured in four sites. Precipitation at Toyotomi town, 6 km from the study sites, is indicated by dotted lines. Site abbreviations: BG – bare ground, RA – *R. alba* sedgeland, MJ – *M. japonica* grassland and SS – unmined bog with dominant *Sphagnum* spp.

Fig. 2 Total nitrogen (TN) concentration and the ratio between NH_4^+ and NO_3^- concentrations (mean ± SD, *n*=3) in peat-pore water throughout the experimental period (October 2005 and October 2007) in four sites, BG, RA, MJ and SS. The concentrations before nitrogen addition, measured in April 2005, are shown by crosses. Site abbreviations: BG – bare ground, RA – *R. alba* sedgeland, MJ – *M. japonica* grassland and SS – unmined bog with dominant *Sphagnum* spp.

Fig. 3 Changes in plant cover (%), species richness per plot and diversity along a nitrogen addition gradient in four sites, BG, RA, MJ and SS. *N.S.*: not significant (by RM-ANOVA, $P<0.05$). Site abbreviations: BG – bare ground, RA – *R. alba* sedgeland, MJ – *M. japonica* grassland and SS – unmined bog with dominant *Sphagnum* spp.

Fig. 4 Changes in dissimilarity along a nitrogen addition gradient at four sites, BG, RA, MJ and SS. Values are mean ± standard deviation. Significant differences among the four sites evaluated with a Tukey *post hoc* test after ANOVA, $P<0.05$. Different letters indicate significant differences between vegetation types. *N.S.*: not significantly different. Site abbreviations: BG – bare ground, RA – *R. alba* sedgeland, MJ – *M. japonica* grassland and SS – unmined bog with dominant *Sphagnum* spp.

Fig. 5 Aboveground biomass of four dominant species in each site with five levels of nitrogen addition. Variables that significantly influence the biomass are indicated in each figure (Tukey *post hoc* test after ANOVA, $P<0.05$). Different letters indicate significant differences between vegetation types. *N.S.*: not significant. Blank figures (N/A) mean that the plants did not become established. Site abbreviations: BG – bare ground, RA – *R. alba* sedgeland, MJ – *M. japonica* grassland and SS – unmined bog with dominant *Sphagnum* spp.

Fig. 6 Aboveground biomass of the four growth forms (forb, grass, shrub and moss) in each site with five levels of nitrogen addition. Variables that significantly influence the biomass are indicated in each figure (Tukey *post hoc* test after ANOVA, $P<0.05$). Different letters indicate significant differences between vegetation types. *N.S.*: not significant. Blank figures (N/A) mean that the plants did not become established. Site abbreviations: BG – bare ground, RA – *R. alba* sedgeland, MJ – *M. japonica* grassland and SS – unmined bog with dominant *Sphagnum* spp.

Fig. 7 Nitrogen standing stocks (g/m^2) in five plant species harvested from *Rhynchospora alba* sedgeland (RA), *Moliniopsis japonica* grassland (MJ) and unmined peatland (SS) in 2007. 'N addition' in the figure indicates significant effect of nitrogen addition on nitrogen standing stock in plants (ANOVA, $P<0.05$). Different letters indicate significant differences between vegetation types (Tukey *post hoc* test, $P<0.05$). *N.S.*: not significant. Site abbreviations: BG – bare ground, RA – *R. alba* sedgeland, MJ – *M. japonica* grassland and SS – unmined bog with dominant *Sphagnum* spp.