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

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1 **Abstract** Nitrogen addition experiments were conducted in three plant communities in a
2 post-mined peatland and on the surrounding original *Sphagnum* bog in northern Japan to compare
3 the effects of nitrogenous fertilization among the plant communities. Gradients of added nitrogen
4 were prepared by adding nitrogen to 1 m × 1 m plots at rates of 0–36 g/m²/yr. These different levels
5 of nitrogen addition were applied to four sites representing a vegetation development
6 chronosequence: bare ground (BG), a *Rhynchospora alba* sedgeland (RA), a *Moliniopsis japonica*
7 grassland (MJ) and the original *Sphagnum* bog (SS). Vegetation was monitored in each plot for
8 three years, and the groundwater level and nitrogen concentration in peat-pore water were
9 monitored in each plot. The nitrogen concentration in peat-pore water increased with increasing
10 nitrogen addition on BG, but was constantly low at the vegetated sites, suggesting the occurrence of
11 nutrient uptake by plants. Species richness decreased as a result of nitrogen addition at the SS site,
12 where plant cover was high. The aboveground biomass of the two common grasses *M. japonica* and
13 *Phragmites communis* was not influenced by nitrogen addition in the post-mined peatland. Grasses
14 and sedges, which produce large underground organs, showed increased biomass as a result of
15 nitrogen addition in the SS plot whereas *Sphagnum* declined. Additionally, forbs declined in the SS
16 plot with increasing nitrogen, most likely due to competition with the increased populations of
17 grasses. Therefore, nitrogen enrichment can promote the dominance grasses, leading to a decrease
18 in mosses and forbs.

19

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

22

23 **Plant nomenclature:**

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

25

1 **Introduction**

2

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

15 Bogs are nutrient-poor habitats that provide safe sites for weakly competitive and slowly
16 growing species (Øien 2004), and are thus sensitive to nutrient enrichment (Van Duren and Pegtel
17 2000). In grassland ecosystems, species richness is reported to be inversely associated with nutrient
18 status (Dupre et al. 2010; Stevens et al. 2004); in other words, their richness declines with
19 increasing nutrient load. To our knowledge, such relationships have not been found in wetlands,
20 although the alteration of nitrogen availability appears to degrade their species composition and
21 community development (Bedford et al. 1999). When nitrogen availability changes, competitive
22 balances between species change with it, accelerating subsequent species turnover (Limpens et al.
23 2003; Schrijver et al. 2011). With increasing nitrogen deposition, nitrogen-demanding species, such
24 as the grass *Molinia caerulea* and the shrub *Betula pubescens*, invade bogs, which is accompanied
25 by a decline in ombrotrophic species (Aerts et al. 1992; Tomassen et al. 2004a). The increased

1 nitrogen levels derived from peat decomposition alter the composition of plant growth forms in peat
2 bogs; shrubs expand at the detriment of graminoid species, for example (Kool and Heijmans 2009).
3 Information on growth forms is used to identify the role of individual plant species in ecosystem
4 functioning and the responses of these species to environmental changes such as nitrogen deposition.
5 Fertilization experiments are useful for understanding the impacts of nitrogen on plant communities
6 and have been undertaken in boreal wetlands, where substantial amounts of peat are accumulated.
7 Such experiments have been conducted especially in Europe and North America (Limpens et al.
8 2011). In Japanese wetlands, nitrogen addition experiments are rare. It is therefore difficult to
9 extrapolate to these valuable ecosystems.

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

1

2 **Materials and methods**

3

4 *Study area and experimental design*

5

6 The field experiment was conducted in the Sarobetsu Mire, northern Hokkaido, Japan (45°06'N,
7 141°42'E, 7 m a.s.l.). Currently, the Sarobetsu Mire is covered by a 2,773 ha *Sphagnum* peat bog,
8 which is approximately 6 m deep (Hotes et al. 2004). From 2005 until 2007, when the surveys were
9 conducted, the mean annual temperature in the town of Toyotomi, 6 km east of the study site, was
10 6.5°C, with monthly averages ranging from -12.6°C in February 2005 to 25.4°C in August 2006
11 (Japan Meteorological Agency 2008). Annual precipitation averaged 955 mm. The snow-free period
12 usually extends from April to November. Over the past 30 years, the maximum snow depth was 114
13 cm.

14 *Sphagnum* peat had been excavated for commercial use at a rate of 3–22 ha/year to a depth of
15 more than 3 m between 1970 and 2003. To compare the effects of nitrogen addition among plant
16 communities, we selected four sites representing a chronosequence. A chronosequence analysis
17 (Nishimura et al. 2009) has indicated that the three communities at the post-mined sites changed
18 from bare ground (BG) to a *Moliniopsis japonica* grassland (MJ) via an intermediate *Rhynchospora*
19 *alba* sedgeland (RA) stage. Three sites were selected to represent post-mined sites (Nishimura et al.
20 2009): BG, mined in 1982 with low plant cover and richness; RA sedgeland, mined in 1975 and
21 with greater than 80 % cover; and MJ grassland, mined in 1970 and with 70–80 % cover. An
22 additional site was established in the surrounding unmined bog (SS) with a predominance of
23 *Sphagnum* spp. (primarily *Sphagnum papillosum* and *S. magellanicum*). At each site, 25 1 m × 1 m
24 plots were set up in the early spring of 2005 at 3–5 m intervals within a 0.25 ha area. Five
25 treatments were applied, differing in nitrogen concentrations and producing nitrogen loads of 0.0,

1 4.5, 9.0, 18.0 and 36.0 g N/m²/yr via nitrogen addition. Because the Sarobetsu Mire is surrounded
2 by agricultural fields, nutrient levels are high in rivers, lakes and the downstream basin near the bog
3 under study (Tachibana et al. 1996). Nitrogen was applied as granular urea, CO(NH₂)₂, an aldehyde
4 condensation compound that slowly and continuously releases ammonium (NH₄⁺) upon hydrolysis.
5 Granular urea was supplied seven times during the snow-free period between April 2005 and
6 October 2006 because plants, *Sphagnum* in particular, utilize NH₄⁺ more than they utilize NO₃⁻ in
7 nutrient-poor, acidic and wet habitats (Jauhiainen et al. 1998; Larcher 2003). To eliminate plot edge
8 effects, urea was also sprayed 20 cm beyond the plots. Each concentration treatment consisted of
9 five replicates.

10

11 *Measurements and sampling*

12

13 In early September of each year, the plant cover was visually estimated for each species in each plot.
14 For this purpose, each plot was divided into 25 cells using a 1 m × 1 m frame. The nomenclature
15 follows Ohwi (1975) for vascular plants, Tagawa (1983) for ferns, and Iwatsuki and Mizutani
16 (1972) for mosses. In the summer of 2006, shoots of the two dominant grasses, *Phragmites*
17 *communis* and *M. japonica*, were counted. Shoots of *P. communis* were counted in each 1 m × 1 m
18 plot, and shoots of *M. japonica* were counted within a 20 cm × 20 cm subplot established in each
19 plot. In 2007 shoots were counted in each plot for *P. communis* and within 40 cm × 40 cm subplots
20 in each plot for *M. japonica* and *Carex middendorffii*. *S. capitulum* density was measured as a
21 surrogate for vitality (Breeuwer et al. 2008). We performed this density measurement by counting
22 the number of capitula in a 20 cm² circle placed randomly within each 1 m × 1 m plot in 2006 and
23 2007. In September 2007 vascular plant aboveground biomass was harvested in a 40 cm × 40 cm
24 subplot except for *P. communis*, which was harvested in the 1 m × 1 m plots. *Sphagnum* biomass
25 was sampled using 100 cm³ cores (20 cm² in surface area and 5 cm in depth) from three locations in

1 each plot. Dead *Sphagnum* tissue, which was brown in colour, was excluded from the biomass
2 samples (Güsewell et al. 2002). The samples of each species were weighed after drying at 65°C for
3 72 h. The vegetation was classified into four growth forms: shrubs, forbs, grasses (including sedges)
4 and mosses (mostly *Sphagnum*; refer to Chapin et al. 1996). The nitrogen content in leaves was
5 measured for the four dominant species (*M. japonica*, *P. communis*, *R. alba* and *C. middendorffii*)
6 and *Sphagnum* spp. The plants were harvested with shears at the ground surface at the three
7 vegetated sites, RA, MJ and SS, if the amount of available biomass was sufficient for harvesting.
8 The nitrogen content of dry shoots (weighing 10–20 mg) was measured with an NC analyser
9 (NC-900, Sumika Chemical Analysis Service, Ltd., Tokyo, Japan). The calibration curve was
10 obtained using acetanilide.

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

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

1 August and October 2007, 20 ml of peat-pore water was extracted from the peat layer to a depth of
 2 20 cm from the surface using a porous tube (DIK-8392, Daiki Rika, Shiga). This water sample was
 3 handled with the same procedures as used for groundwater. Concentrations of NH_4^+ , NO_3^- and
 4 NO_2^- in the water were measured with an automatic water analyser (QuAATro TNTP Multi, Bran
 5 Luebbe Co., Tokyo).

6

7 ***Data handling and statistical analysis***

8

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

10
$$\text{PD} = \left\{ 1 - \frac{\left(2 \times \sum_{i=1}^n \min(x_i, y_i) \right)}{\sum_{i=1}^n (x_i + y_i)} \right\} \times 100,$$

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

15
$$H' = - \sum_{i=1}^n p_i \ln p_i,$$

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

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

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

9

10 **Results**

11

12 ***Water chemistry in peat***

13

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

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

1 similar in MJ and SS. BG showed the lowest water level and responded to rainfall more rapidly than
2 any other vegetated site. Nitrogen addition affected the TN concentration at the BG and MJ sites
3 (Fig. 2). The TN concentration in BG and MJ plots significantly increased with nitrogen addition
4 (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: $d.f.=3$,
5 $F=9.38$, $P<0.001$; PM: $d.f.=3$, $F=3.42$, $P=0.030$). Additionally, TN was higher in the BG plot than
6 at the other sites in all seasons. The TN concentration at the RA and SS sites differed significantly
7 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
8 the NH_4^+ and NO_3^- concentrations was not significantly affected by nitrogen addition at any site. In
9 the RA plot, this ratio fluctuated significantly among months, as shown by the repeated-measures
10 data ($d.f.=3$, $F=3.05$, $P<0.001$).

11

12 ***Responses of plant communities to nitrogen addition***

13

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

22 In the RA plot, the plant cover ranged from 40 % to 80 %, including eight forbs, three grasses,
23 four sedges and five mosses. In the RA control plots, the most dominant species was *R. alba*, with a
24 total cover of 46–61 %; the total cover of *C. middendorffii*, *S. papillosum* and *M. japonica* was
25 5–16 % in each case. The plant cover varied significantly over time ($d.f.=2$, $F=13.20$, $P<0.001$), and

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

4 In the MJ control plots, *M. japonica* showed a total cover of 37–55 % and was most dominant.
5 The composition of growth forms was similar to that in the RA plot (i.e. nine forbs, three grasses,
6 four sedges, five shrubs and three mosses). In the MJ plot without nitrogen addition, *C.*
7 *middendorffii* and *P. communis* were dominant, with more than 10 % cover, and *R. alba*, *S.*
8 *papillosum* and *Myrica gale* were also present. *M. gale* is a low shrub and showed a total cover of
9 approximately 10 %. Among the forbs, *Lobelia sessilifolia* and *Hosta rectifolia* were the most
10 frequent, showing a low total cover of 2–3 %. Nitrogen addition did not influence the plot cover
11 ($d.f.=4$, $F=0.88$, $P=0.49$), species richness ($d.f.=4$, $F=0.88$, $P=0.50$) or diversity ($d.f.=4$, $F=0.11$,
12 $P=0.98$; Fig. 3).

13 In the original bog (SS), there were 12 species of forbs, one grass, one sedge, six shrubs and
14 five mosses. In the control plots, *S. papillosum* was the most common, with a cover of more than
15 80 %. *S. magellanicum* was also well established, with a cover of 8–14 %. Shrub richness in the SS
16 plot was the highest of the four sites and was represented by *Empetrum nigrum*, *M. gale* and
17 *Vaccinium oxycoccus*. Among vascular plants, *C. middendorffii* had the highest cover, ranging from
18 20–36 % in the control, whereas the cover of the other vascular plants was less than 10 %. It is
19 probable that mosses took up more nutrients than vascular plants in the SS plot because of the
20 dominance of *Sphagnum*. In contrast, the relative role of mosses in nutrient uptake was most likely
21 less marked in the RA and MJ plots. The plant cover was significantly affected by nitrogen addition
22 ($d.f.=4$, $F=22.59$, $P<0.001$) and the year (repeated-measures analysis, $d.f.=2$, $F=78.06$, $P<0.001$),
23 with a significant interaction between the two factors ($d.f.=8$, $F=15.28$, $P<0.001$; Fig. 3), indicating
24 that the response of the plant cover over the experimental period differed among the nitrogen
25 addition treatments. Species richness in the SS plot differed significantly as a result of nitrogen

1 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,
2 diversity at the SS site responded significantly to nitrogen addition ($d.f.=4$, $F=3.00$, $P=0.043$) and
3 fluctuated annually ($d.f.=2$, $F=6.79$, $P<0.001$; Fig. 3). Overall, the SS site would be more
4 susceptible to a change in nitrogen conditions than the vegetated sites. Accordingly, the species
5 composition at the SS site would tend to change easily.

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

10

11 ***Responses of biomass to nitrogen addition***

12

13 The analysis of aboveground biomass harvested in the third year (Fig. 5) showed that nitrogen
14 addition did not influence the biomass of the two grasses *P. communis* and *M. japonica* in any of the
15 plots. With increasing nitrogen addition, *R. alba* tended to decrease in RA and MJ plots, but these
16 results were not statistically significant (RA: $d.f.=4$, $F=1.50$, $P=0.24$; MJ: $d.f.=4$, $F=0.19$, $P=0.94$).
17 *C. middendorffii* showed increased biomass in the SS plot with increasing nitrogen addition ($d.f.=4$,
18 $F=3.22$, $P=0.03$). This species tended to show increased biomass in the RA and MJ plots at the
19 nitrogen addition rate of 36 g/m²/yr, although the results were not statistically significant (RA:
20 $d.f.=4$, $F=0.66$, $P=0.63$; MJ: $d.f.=4$, $F=0.25$, $P=0.91$). At the post-mined sites, nitrogen addition did
21 not influence the biomass of any of the dominant species.

22 The responses of the biomass of each growth form to nitrogen addition differed among the sites
23 (Fig. 6). In particular, nitrogen addition increased grass biomass ($d.f.=4$, $F=3.65$, $P=0.02$) in the SS
24 plot, but completely eliminated mosses ($d.f.=4$, $F=42.5$, $P<0.001$). The addition of 36 g N/m²/yr to
25 the SS plot decreased the biomass of mosses to near zero. Nitrogen addition caused the biomass of

1 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
2 decrease in the MJ plot (not significantly; $d.f.=4, F=1.95, P=0.14$) whereas no effect of nitrogen
3 addition on forb biomass was found in the RA plot ($d.f.=4, F=0.85, P=0.51$). No statistically
4 significant effect of nitrogen addition on shrub biomass was found in the RA or SS plots (RA: $d.f.=4,$
5 $F=1.06, P=0.40$; SS: $d.f.=4, F=0.85, P=0.51$). In the MJ plot, the only effect of nitrogen addition on
6 shrub biomass was increased biomass at the addition rate of $18 \text{ g/m}^2/\text{yr}$ ($d.f.=4, F=2.85, P=0.05$).

7 Capitulum density of *Sphagnum* increased with increasing nitrogen up to $18 \text{ g/m}^2/\text{yr}$ at all of
8 the sites from 2006 to 2007, but decreased at the nitrogen addition rate of $36 \text{ g/m}^2/\text{yr}$ in the SS plot
9 (Table 2), showing that vitality decreased as a result of excessive nitrogen addition. *Myrica*
10 *japonica* showed the highest shoot density in the RA plot except at the nitrogen addition rate of 9.0
11 $\text{g/m}^2/\text{yr}$ and showed a low shoot density in the MJ plot, indicating that this species developed well
12 in the transitional *R. alba* grassland and then declined. The shoot density of *P. communis* in the RA
13 plot was not changed by nitrogen addition and decreased slightly in the MJ plot with increasing
14 time, indicating that nitrogen addition had the least influence on *P. communis*. Nitrogen addition
15 tended to increase the shoot density of *C. middendorffii* in 2007.

16

17 ***Nitrogen concentration in plants***

18

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

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

4 5 **Discussion**

6 7 *Community responses to nitrogen addition*

8
9 One of the objectives of ecological restoration is to accelerate ecosystem recovery, as evaluated in
10 terms of community structure and diversity. After peat mining, the spontaneous recovery of a
11 *Sphagnum* bog requires more than three decades and occasionally fails (Nishimura et al. 2009).
12 Nitrogen addition did not influence the plant communities at the post-mined sites but decreased the
13 plant cover, species richness and diversity at the unmined site. The most dominant vascular plants
14 were large grasses and sedges. The decrease in species richness resulted primarily from a decrease
15 in forbs, particularly at the SS site. Nitrogen fertilization enhances shoot competition via height
16 growth rather than via root competition in peatlands (Limpens et al. 2003; Güsewell 2005; Kool and
17 Heijmans 2009). With increasing nitrogen in the Sarobetsu Mire, grasses appeared to suppress the
18 growth of forbs through shoot competition.

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

1 2011).

2

3 ***Relationships between growth form and nitrogen addition***

4

5 Grass biomass increased as a result of nitrogen addition in the SS plot whereas mosses and forbs
6 decreased. Nutrient requirements differ among growth forms (i.e. forbs, grasses and shrubs) and
7 between habitats (i.e. bogs and fens; Bowman et al. 1995). Two shrubs, *Vaccinium myrtillus* and *V.*
8 *vitis-idaea*, which are dominant in bogs, show optimal growth if moderate nitrogen is supplied and
9 decreased growth if excess nitrogen is supplied (Makipaa 1999). Moreover, nutrient addition greatly
10 increases aboveground biomass by stimulating plant productivity and by shifting species
11 composition from slow-growing species to productive species such as dwarf shrubs like *Betula*
12 *nana* (Mack et al. 2004). Accordingly, grasses and shrubs increased as *Sphagnum* decreased in the
13 SS plot. By contrast, although shrub biomass in the MJ plot showed a partial increase at the
14 nitrogen addition rate of 18 g/m²/yr, but at no other nitrogen addition rate, the biomass of grasses
15 and shrubs did not change in the post-mined peatlands (i.e. the RA and MJ sites). Because less
16 *Sphagnum* develops in post-mined peatlands, other stressors restrict the growth of grasses and
17 shrubs (Koyama and Tsuyuzaki 2010).

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

1 2001), suggesting that nitrogen addition indirectly restricts the growth of *Sphagnum*.

2

3 ***Responses of vascular plants to nitrogen addition to peat***

4

5 Bare ground is subject to drought, strong wind and ground surface instability, all of which restrict
6 seedling establishment (Eckstein and Donath 2005; Koyama and Tsuyuzaki 2010). Early
7 recolonization of mined peatlands can reduce these stresses. Solutes in groundwater are transported
8 upward to the bare ground by capillary action in peat and get concentrated by evaporation
9 (Cobbaert et al. 2004). In addition, available nitrogen is enriched relative to the original bog in
10 post-mined peatlands by oxidation under aerobic conditions (Andersen et al. 2006). In a post-mined
11 peatland, the ammonium concentration becomes high due to the removal of *Sphagnum*, which
12 requires ammonium nitrogen rather than nitric nitrogen (Jauhiainen et al. 1998; Tomassen et al.
13 2004b). Nitrogen concentrations were lower at the vegetated sites, particularly in the SS plot, than
14 on BG, indicating that the plants absorbed nitrogenous ions from peat-pore water.

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

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

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

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

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

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

6
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Fig. 1 Seasonal fluctuations in water level (mean \pm 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 \pm 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 \pm 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.