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Microbial dynamics and litter decomposition under a changed climate in a Dutch heathland

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ABSTRACT

Climate change scenarios predict changes in temperature and precipitation. The effect of a modest temperature increase and repeated summer droughts on the rate of litter decomposition and microbial biomass dynamics was studied by a field scale manipulation experiment at a phosphorus (P) deficient dry heathland ecosystem in the Netherlands. Retractable covers were used to create artificial nighttime warming or prolonged summer drought in the experimental plots. The warming treatment initially enhanced litter mass loss and two consecutive years of summer drought retarded litter decomposition rate. Microbial carbon (C), nitrogen (N) and P immobilization was affected by the warming treatment as well as by the drought treatment. Enhanced temperatures resulted in increased microbial biomass C during the first half year of incubation, whereas the first drought treatment significantly retarded microbial N and P immobilization. The delayed net microbial N and P immobilization in the drought plots prevented net N and P mineralization. After 1 year microbial biomass C, N and P were significantly higher in the drought plots, probably as a result of availability of new substrate caused by the drying and rewetting process. Although microbial biomass was higher in the drought plots, the microbial C/N ratio was equal to the control and varied between 6 and 8. This suggested that in both the control and drought plots, the microbial community was dominated by bacteria at the longer term. Both treatments reduced net P mineralization and together with decreased foliar P concentrations this indicated the progressive importance of P limitation in restraining plant growth in this N saturated ecosystem.

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1. Introduction

Microbial activity plays an important role in decomposition of organic matter and the immobilization and mineralization of nutrients. Numerous factors, such as soil mineral sources, amount and quality of soil organic matter, soil texture, atmospheric input, water content, soil temperature and freezing, affect microbial activity (Panikov, 1999). These factors are considered to be dynamic and changing in the

course of hours, days and months. Ecosystem functioning is adapted to this daily, monthly and seasonal variability in environmental conditions. However, changes in mean global air temperature and precipitation patterns are predicted, resulting in increased temperatures of between 1.4 and 5.8 °C over this century as well as more severe droughts and floods (Houghton et al., 2001). This climate change will affect key ecosystem processes such as plant growth, plant net photosynthetic and transpiration rate, plant nutrient uptake

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(Llorens et al., 2004; Peñuelas et al., 2004), microbial activity (Sowerby et al., 2005), and nutrient mineralization, immobilization and leaching (Jonasson et al., 1999; Rustad et al., 2001; Schmidt et al., 2004). Microbial nutrient mobilization is an important process for the regulation of plant growth in nutrient-deficient ecosystems (Harte and Kinzig, 1993; Jonasson and Michelsen, 1996). The impact of climate change might alter microbial dynamics, decomposition of organic matter and mineralization of nutrients. Together these changes may have a strong impact on the functioning of nutrient-poor ecosystems. At the Dutch heathland area 'Oldebroekse heide' climate conditions have been manipulated since May 1999 by nighttime warming and summer drought (Beier et al., 2004). The site has a highly weathered, acid sandy soil, with very low phosphorus (P) concentrations, whereas because of high nitrogen (N) deposition N leaching is high (Schmidt et al., 2004). Decomposing litter is an important nutrient pool for plants and microorganisms but microbial biomass itself is also considered as an important nutrient pool (Kouno et al., 2002). The dominating dwarf shrub at this site, *Calluna vulgaris* (L.) Hull. produces P poor litter, with an N/P ratio of >16, indicating that P is the most important nutrient limiting vegetation growth (Koerselman and Meuleman, 1996). In order to study the effect of changes in climate on (1) the decomposition rate of *C. vulgaris* litter and (2) the size and dynamics of the microbial biomass decomposing this litter and (3) the subsequent nutrient mineralization, we performed a litterbag incubation experiment at this site.

2. Materials and methods

2.1. Site description and treatment

The experiment was conducted at a Dutch heathland area called 'Oldebroekse heide' located in the center of the Netherlands (52°24'N, 5°55'E). The site is dominated by the perennial woody dwarf shrub *C. vulgaris* (95% groundcover). The soil is a nutrient-poor, well-drained, acid sandy Haplic Podzol (FAO, 1998), with a mormoder humus form (Green et al., 1993). The site experiences high N deposition (20 kg N ha⁻¹ year⁻¹) and further site characteristics are presented in Table 1.

Nine experimental plots of 5 m × 4 m each were established in homogeneous areas within the site. Each plot was randomly assigned a treatment: control (C), heating (H) and prolonged drought during the growing season (D), so that three replicate plots per treatment were present. Around each plot, a light scaffolding structure was built of galvanized steel tubes covered by thin plastic sleeves to prevent contaminants leaching into the plot. In the heating plots, this frame supported a retractable, reflective curtain made of strips of infrared reflective material bound into a high-density polyethylene mesh. A small motor activated by a light sensor draws the curtain over the vegetation at night and triggers its removal again during the daytime. This treatment reduces the loss of infrared radiation from the surface at night.

A tipping bucket rain sensor activated the removal of the curtain at night to enable rain to enter the plot. Over the drought plots, the retractable curtain was made of transparent polyethylene plastic. During 2 months in the growing season

Table 1 – Site characteristics 'Oldebroekse heide' (Emmett et al., 2004), N deposition was determined with a funnel above the vegetation

Location	52°24'N, 5°55'E
Altitude (m)	25
Yearly mean air temperature (°C)	10.1
Precipitation (mm year ⁻¹)	1042
N deposition (kg ha ⁻¹ year ⁻¹)	20
Vegetation	<i>Calluna vulgaris</i>
Above-ground C (g C m ⁻²)	584
Soil type	Haplic Podzol
Humus form	Mormoder
Organic layer	
Depth (cm)	0–4
pH	3.7
C/N (g g ⁻¹)	22.5
Organic matter (g kg ⁻¹)	650
Bulk density (g cm ⁻³)	0.11
Organic rich mineral horizon	
Depth (cm)	4–16
pH	3.8
Organic matter (g kg ⁻¹)	33
Bulk density (g cm ⁻³)	1.41

(generally June and July), the rain sensor activated the motor to extend this cover over the plots once rain was detected and removed the cover when the rain had stopped. Monitoring started in December 1998 (pre-treatment) and the treatments started in May 1999.

Temperature of the air and soil was measured by installation of temperature sensors in the air (20 cm above the soil surface) and in the topsoil (0, –5 and –10 cm). The temperature sensors were PT100 thermistors (Campbell Scientific). One temperature sensor per depth was placed in one control and one adjacent temperature plot. Temperature was measured every 20 min across all seasons. In the topsoil, warming was on average 0.5 °C (–10 to 0 cm). In the air (20 cm above the soil) an average temperature increase of 0.7 °C during the night (04:00 h) was observed, which decreased gradually during the day to 0 °C at 16:00 h (Beier et al., 2004). Precipitation input to each plot was collected two or three weekly by 1 rain gauge (Ø 21.2 cm) per plot placed above the height of the vegetation. The 2-month summer drought treatment reduced precipitation in the growing season (May to September) with 45% compared to control in 1999, 51% in 2000, 43% in 2001, 43% in 2002 and 70% in 2003. Water content in the mineral soil was measured daily at 0–10 cm and 0–45 cm soil depth in 1 control, 1 temperature and 1 drought plot with time domain reflectometry (TDR, Topp et al., 1980). In the other 6 plots soil moisture content was measured two or three weekly, also with TDR. Soil water content in the top 10 cm of the mineral soil was reduced by 82% at the peak of the drought treatment in 1999 and 2000 (Beier et al., 2004), by 80% in 2001, 83% in 2002 and 73% in 2003. Water content of the litter layer, measured in litterbags, is presented in Table 2. Heating did not result in a reduction in litter water content, except at 62 days, when water content was slightly reduced. During the drought treatment (day 160) litter water content was significantly reduced. Further details on the design and treatment effects can be found in Beier et al. (2004).

Table 2 – The effect of the treatments on litter moisture content, measured in the litterbags

	62 days			160 days			365 days			628 days		
	C	H	D	C	H	D	C	H	D	C	H	D
Litter moisture content (%)	12.5	11.4*	10.4*	51.9	47.8	8.9*	64.6	66.1	66.2	76.6	75.3	75.3

Data are means per treatment ($n = 27$). The effect of treatment was tested by a paired two samples for means t-test. Significant treatment effect is indicated with * $P < 0.05$.

2.2. Litterbag experiment

Ready-to-fall *C. vulgaris* litter, consisting of flowers, shoots and branches (under 1.5 mm in diameter and under 20 mm in length for shoots; under 40 mm in length for branches), was collected outside the plots in autumn 2001 by gently shaking the shrubs. The collected litter was bulked, left in the open air in the laboratory at approximately 20 °C to let macro fauna escape, uniformly mixed and stored in plastic bags at 3 °C. Bulked litter consisted of 56% flowers, 27% shoots and 4% branches (on a mass basis); the remainder consisted of small unidentifiable particles. Initial chemical composition of the bulked litter was 543 g C kg⁻¹, 11.81 g N kg⁻¹, 0.61 g P kg⁻¹, 3.53 g Ca kg⁻¹ and 0.20 g Mn kg⁻¹. The initial C/N and N/P ratio (kg kg⁻¹) was respectively 46 and 19 and the lignin and cellulose concentration was respectively 393 and 563 g kg⁻¹. Litterbags (9.0 cm × 9.0 cm, with a mesh size of 1 mm²) were filled with 5.00 g of the litter and placed beneath *C. vulgaris* plants at 3 different areas in each of the nine plots (3 control plots, 3 heating plots and 3 drought plots). In total 324 litterbags were placed in the experiment (36 litterbags per plot). Litterbags were placed in the field at 7 February 2002 and 9 replicate bags per plot (3 per area) were retrieved at after 62 days (10 April 2002), 160 days (17 July 2002), 382 days (24 February 2003) and 628 days (28 October 2003). A set of 27 litterbags was brought to the field on 7 February 2002 and taken back the same day. The contents of these litterbags were weighed and taken as the initial weight of the litterbags. Drought treatment, during the incubation experiment, was from day 100 to day 184 (18 May to 10 August 2002) and day 480 to day 540 (2 June to 1 August 2003). Initial microbial and chemical characterization of the litter material was performed on 9 replicate samples of the 3 °C stored material. This initial characterization was taken as day 0.

Data of dry litter remaining in the litterbags was fitted to a single negative exponential decay model Eq. (1) (Hunt, 1977):

$$X_t = X_0 \times e^{-kt} \quad (1)$$

where X_t = mass litter left at time t (g); X_0 = mass initial amount of litter (g); t = time (years); k = fractional weight loss (year⁻¹).

2.3. Chemical analysis

At each collection day 3 litterbags from each of the 3 incubation areas in the plots were collected (total 9 bags per plot; 27 per treatment) and brought to the laboratory. Litterbags were cut open and ingrown mosses were removed. The total content was weighed, homogenized and moisture content was determined by drying a sub sample of approxi-

mately 2 g at 70 °C for 48 h. The 3 litterbags retrieved from the same incubation area in one plot were pooled in Mason jars that could be closed airtight and the samples were left to rest for one night at 5 °C. This was done to make sure that the CO₂ respiration rate measurement the next day was not influenced by the homogenizing and pooling of the samples. The next day the jars were aerated and closed airtight. A septum in each jar lid facilitated the removal of a sample of the accumulating headspace CO₂ using a gas-tight syringe. The headspace was sampled after 0, 3 and 6 h. The collected headspace gas was immediately injected in a Carlo Erba gas chromatograph to quantify the CO₂ concentration. The difference between the headspace CO₂ concentration divided by the time between measurements was considered the respiration rate (Witkamp and Frank, 1969). Then the litter held in the bottles was split into several sub samples for further analysis. Microbial biomass C and N were determined by fumigating a moist sample corresponding to 1.5 g dry weight for 24 h with ethanol free CHCl₃. Samples were then extracted with 50.0 ml 0.5 M K₂SO₄ (for 1 h) (Brookes et al., 1985; Vance et al., 1987). At the same time a non-fumigated sub sample was also extracted with 0.5 M K₂SO₄. Extractions were analyzed for dissolved organic carbon (DOC), total N, N-NH₄⁺, N-NO₃⁻ and N-NO₂⁻ on a Skalar continuous flow auto-analyzer, so dissolved organic nitrogen (DON) concentrations could be calculated. Microbial biomass C was estimated as the difference between the DOC concentration of the fumigated and unfumigated extracts. Microbial biomass N was estimated as the difference between the summed DON, N-NH₄⁺, N-NO₃⁻ and N-NO₂⁻ concentrations of the fumigated and unfumigated extracts. Because of analytical problems DON was not measured at day 0. DON is part of the lysed microbial cells after fumigation, but the size of the microbial biomass C at day 0 was very small, indicating that DON could never have been a substantial part of the lysed microbial N. An extractability of 0.45 was assumed when calculating the microbial C and N (Wu et al., 1990).

Available P (P_i) was analyzed by shaking a moist sub sample corresponding with 0.5 g of dry litter with 50.0 ml 0.5 M NaHCO₃ (pH 8.5 for 30 min) (Olsen et al., 1954). The resulting solution was filtered and solution P concentrations were determined colorimetrically by the ammonium molybdate ascorbic acid method as described by Murphy and Riley (1962). Soil microbial biomass P was determined by the fumigation extraction method as described by Brookes et al. (1982). Here a moist sub sample corresponding to 0.5 g dry litter was fumigated for 24 h with ethanol free CHCl₃ and extracted with 50.0 ml 0.5 M NaHCO₃ (pH 8.5). P concentration was also determined colorimetrically by the ammonium molybdate ascorbic acid method. Microbial biomass P was calculated as the difference between the P concentration of the fumigated and unfumigated extracts, an extractability of 0.40 for P was

Fig. 1 – Litter mass remaining as percentage of initial mass for the control and heated plots (a) and the control and drought plots (b). Significant treatment effect at one sampling date is indicated by * $P < 0.05$ and # $P < 0.1$. A significant difference ($P < 0.05$) with the previous sampling date within one treatment is indicated with C (for control), T (for heated) and D (for drought). Error bars indicate standard deviation ($n = 3$). (●) control; (◆) heated; (○) drought; (—) drought period.

assumed (Brookes et al., 1982). Total C and N content of the samples was measured by a CNS analyzer (Vario EL analyzer, Elementar). Total P content was measured on ICP-OES (Optima 3000XL, Perkin-Elmer) after an $\text{HNO}_3 + \text{HCl}$ digestion (4.0 ml HNO_3 65%, 1.0 ml HCl 37% and 1.0 ml H_2O) of an 250.0 mg sample (grinded and homogenized (<0.2 mm)) in a microoven (Multiwave, Anton Paar).

2.4. Statistics

The data was analyzed with a linear mixed-effects model using the formulation described in Laird and Ware (1982) but allowing for nested random effects. A (simpler) analysis of variance on the basis of a complete randomized block design was not allowed since the assumption of homogeneous variance and covariance across time was not met. In the linear mixed model, treatment is a fixed factor while time is a random factor. The effect of the warming or drought treatments at distinct sampling dates, as well as effects within a single treatment between different sampling dates are tested with a Wilcoxon-test. Differences in means were called significant when $P < 0.05$ or weakly significant when $P < 0.1$. Data are reported as means $\pm$ standard deviation. The analyses were performed in R, using the library nlme (see Venables and Ripley, 2002).

3. Results

The time-effect was highly significant ($P < 0.001$) for each of the distinct processes. Hence it is not separately noticed in the subsequent sections.

3.1. Decomposition

The percentage of litter remaining at the final sampling date amounted to 61.9 (± 4.8)% for the litter in the control plot, 62.0 (± 4.3)% for the litter in the heated plot and 70.0 (± 7.5)% for the litter in the drought plot (Fig. 1). No significant effect of the heating treatment on the cumulative mass loss at the final sampling date was found. However the heating treatment significantly reduced the percentage remaining of initial mass after 62 days and weakly significantly after 160 days (Fig. 1a).

In the drought plots mass loss was significantly reduced by the first drought treatment and was even further reduced by the following drought treatment in summer 2003 (Fig. 1b). Overall, the drought treatments reduced weight loss weakly significant. The fractional weight loss constant k Eq. (1) was 0.32 (± 0.03) year^{-1} for the control plots, 0.32 (± 0.03) year^{-1} for the heated plots and 0.27 (± 0.03) year^{-1} for the drought plots. The k value from the drought plots was significantly lower than the k values from the control and heated plots.

3.2. Microbial CO_2 respiration

As all ingrown mosses were removed from the litterbag material before the measurement of the CO_2 respiration rate, the measured CO_2 originated from microbes present in the litter material. Overall, the mixed-effect model showed a weakly significant result for the drought treatment only (a reduction in respiration by -0.34 ± 0.20 $\text{g (kg dry litter day)}^{-1}$). The CO_2 respiration rate at 62 days was the same as the CO_2 respiration rate of the initial material (Fig. 2), but had increased in the control and heated plots the next sampling date (160 days). At this date, CO_2 respiration rate was significantly reduced by the drought treatment, however the

Fig. 2 – CO_2 respiration rate ($\text{g kg}^{-1} \text{ day}^{-1}$). Significant treatment effect at one sampling date is indicated by * $P < 0.05$. A significant difference ($P < 0.05$) with the previous sampling date within one treatment is indicated with C (for control), T (for heated) and D (for drought). Error bars indicate standard deviation ($n = 3$).

following sampling date (at 382 days) it had completely recovered and exceeded CO_2 respiration rate in the control and heated plots. No differences in CO_2 respiration rate between the control and heated plots was observed and CO_2 respiration rate in winter (382 days) was equal to respiration rate in summer (160 days). At the final sampling date (628 days), CO_2 respiration in the control and heated plots had not changed with respect to 382 days, however CO_2 respiration in the drought plots had significantly declined.

3.3. Microbial biomass C, N and P

Overall, the C content of the microbial biomass increased weakly significant in the drought treatment ($2.26 \pm 1.32 \text{ g (kg dry litter day)}^{-1}$). There were no significant overall effects for microbial N and P, or for the microbial C/N ratio. Microbial biomass C increased in all plots during the first incubation period, and a significant effect of the heating and drought treatment at 62 days was found (Fig. 3). At this sampling date microbial biomass C was highest in the heated plots and slightly higher in the drought plots than in the control. The drought treatment had not started yet at this sampling date, so differences in microbial biomass C in the drought plots at this point could be ascribed to the effects of the summer drought treatments in 1999, 2000 and 2001. These treatments were performed before the installation of the litterbags, but may have an effect on the colonization of the litterbags by microorganisms. At 160 days, during peak drought, microbial biomass C had increased further and again a significant positive effect of the heating and drought treatment was found. The next collection date (382 days), microbial biomass C was still significantly enhanced in the drought treatment. At the final sampling date (628 days), microbial biomass C had

decreased significantly in all plots, but remained significantly higher in the drought plots.

Microbial biomass N and P did not increase during the first incubation period (0–62 days), but increased in the second incubation period (62 days to 160 days) (Fig. 3b and c). At 160 days, during the first drought treatment, microbial biomass N and P in the drought plots were significantly less than in the control plots. At 382 days, microbial biomass N and P in the drought plots had increased significantly and exceeded microbial biomass N and P in the control plots, while microbial biomass N and P in the heated plots was equal to the control. On the final sampling date (628 days), both microbial biomass N and P were higher in the drought plots and microbial biomass P was higher in the heated plots.

Microbial C/N ratio increased rapidly from the start of the experiment, with higher C/N ratios in the heated and drought plots at 62 days (Fig. 3d). At 160 days, the C/N ratios had decreased significantly, although the C/N ratio in the drought plots remained higher as in the control and heated plots. On the last two sampling dates microbial C/N ratio was equal in all plots, with an average value of 7.

3.4. Total C, N and P

For the total C, N and P concentration there was (next to a time effect as mentioned previously) only a highly significant interaction effect between time and the drought treatment (for total C enhancing the time effect, while for total N and P diminishing the effect).

The total C concentration in the litter decreased steadily from $544 (\pm 2) \text{ g kg}^{-1}$ initially to $520 (\pm 7) \text{ g kg}^{-1}$ in the control, $519 (\pm 4) \text{ g kg}^{-1}$ in the heated and $509 (\pm 26) \text{ g kg}^{-1}$ in the drought plots at the final sampling date (628 days) (Wilcoxon signed rank test: $P < 0.001$) (Fig. 4a). No significant differences

Fig. 3 – Microbial biomass (a) C, (b) N and (c) P (g kg^{-1}) and (d) microbial C/N ratio (g g^{-1}). Significant treatment effect ($P < 0.05$) at one sampling date is indicated by *. A significant difference ($P < 0.05$) with the previous sampling date within one treatment is indicated with C (for control), T (for heated) and D (for drought). Error bars indicate standard deviation ($n = 3$).

Fig. 4 – Total (a) C, (b) N, (c) P concentrations (g kg^{-1}) and (d) total N and P (g m^{-2}) in the control, heated and drought plots. Significant treatment effect ($P < 0.05$) at one sampling date is indicated by + for the heated plots and * for the drought plots. A significant difference ($P < 0.05$) with the previous sampling date within one treatment is indicated with C (for control), T (for heated) and D (for drought). Error bars indicate standard deviation ($n = 3$). (●) control; (●) heated; (○) drought; (—) drought period.

in total C concentration among the control, heated and drought plots at all collection dates were found. Total microbial biomass C concentration at 628 days was $1.6 (\pm 0.2)\%$ of total C concentration in the control and heated plots and $2.0 (\pm 0.3)\%$ of total C concentration in the drought plots.

Total N concentration increased in the control and heated plots from day 0 to day 628 (Wilcoxon signed rank test: $P < 0.001$). In the drought plots total N concentration also increased from day 0 to day 382 but remained constant from 382 to 628 days (Fig. 4b). No significant differences in total N concentration among the control, heated and drought plots were observed, except at 160 days (during the actual drought treatment), at this date total N concentration in the drought plots was lower. After the drought treatment in 2002 total N concentration increased to the level of total N in the control and heated plots. At the final sampling date respectively $9.8 (\pm 1.0)\%$, $10.8 (\pm 0.9)\%$ and $11.9 (\pm 2.3)\%$ of total N was immobilized in microbial biomass in the control, heated and drought plots.

In the first incubation period, total P concentration increased in the control and heating treatment. From 62 days to 160 days total P concentration increased further in both the control and heated plots, while total P concentration in the drought plots remained significantly lower, which was also found for total N concentration (Fig. 4c). At 382 days, total P concentration had declined significantly in the control plots. In the drought plots total P concentration increased from day 62 to day 382. From day 382 to 628 days the total P concentration remained constant in all plots. At 628 days, $81.4 (\pm 8.5)\%$ of total P in the control plots was incorporated in

microbial biomass and $88.5 (\pm 14.0)\%$ in the heated and drought plots.

Because the changes in total N and P concentration in time cannot be used to signify N or P mineralization, we converted the total N and P concentration to the absolute amount in g m^{-2} by multiplying the concentration with the weight of the sample and dividing this by the size of the litterbag ($9.0 \text{ cm} \times 9.0 \text{ cm}$). Total N and P in g m^{-2} declined significantly from day 0 to day 628 in the control and heated plots (Wilcoxon signed rank test: $P < 0.001$), indicating net N and P mineralization (Fig. 4d). In the control plots 2.523 g N m^{-2} and 0.324 g P m^{-2} and in the heated plots 1.792 g N m^{-2} and 0.230 g P m^{-2} was mineralized during that period. Net N mineralization occurred mainly from day 160 to day 628 and net P mineralization occurred mainly from day 160 to day 382. In the drought plots no net N and P mineralization occurred (an effect which occurs in the mixed-model as a time–drought interaction). At day 628, total accumulated N in the control, heated and drought plots amounted respectively to $20.103 \text{ g N m}^{-2}$, $20.835 \text{ g N m}^{-2}$ and $21.991 \text{ g N m}^{-2}$. Total accumulated P amounted respectively to 0.830 g P m^{-2} , 0.924 g P m^{-2} and 1.020 g P m^{-2} .

3.5. N and P availability

The N-NO_3^- concentration in all litterbags was very low: the majority of the samples ($>70\%$) had N-NO_3^- concentrations below the detection limit ($<1.98 \text{ mg N kg}^{-1}$). In the samples that had measurable concentrations no significant differences between plots or sampling dates were observed (data not shown). The N-NH_4^+ concentration only increased in the

control and heated plots during the first two incubation periods from $0.024 (\pm 0.002) \text{ g N kg}^{-1}$ to $0.101 (\pm 0.10) \text{ g N kg}^{-1}$ in the control and heated plots (data not shown). This corresponds to $0.48 \text{ mg N kg}^{-1} \text{ day}^{-1}$, which is 10% of N mineralization rate in the organic layer (Emmett et al., 2004). At 382 days, the N-NH_4^+ concentration had declined significantly in the control plots and for the remainder of the experiment N-NH_4^+ concentrations in the litterbags remained constant and no significant differences between the treatments were found.

Negligible amounts of P were present as inorganic P (P_i) in relation to P that is immobilized in the microbial biomass (data not shown), 1.5–5.1% of total P was present as P_i . While 52% (at 160 days) and 86% (at 628 days) of total P was immobilized in microbial biomass. From the start of the incubation there was a slight increase in P_i , although it was not affected by the treatments and P_i concentration did not change during the remainder of the experiment, except at 628 days, when P_i had decreased significantly in the control and heated plots.

4. Discussion

The continuously enhanced temperature increased litter mass loss during the first two incubation periods (0–62 days and 62–160 days). This fits within the findings by Liski et al. (1999), who concluded that the rate of decomposition responded less to temperature changes the more decomposed (more recalcitrant) the material is. Litter mass loss in the drought plots did not catch up with litter mass loss in the control plots in between and after the drought treatments, resulting in decreased litter decomposition rate in the drought plots. This agrees with results from Emmett et al. (2004) who found a decrease in soil CO_2 respiration rate in both summer and winter, so including periods outside the experimental drought treatments, measured bi- or three weekly in permanent chambers installed at the field location.

In the first decomposition period microorganisms immobilized C before N and P, which resulted in very high microbial C/N ratios (Fig. 3). The easily decomposable starches and sugars that are decomposed during the initial phase of litter decomposition are mainly composed of C, O and H atoms (de Leeuw and Largeau, 1993), which could explain the initial gain of C before N and P. The size of the microbial biomass N and P was positively affected by the heating treatment at 628 days. Before this period no response or even a slight negative response at 62 days for microbial biomass N was found. In previously published work the response of microbial nutrient transformation rates to heating was variable; increased rates have been reported (Rustad et al., 2001; Schmidt et al., 2002; Jonasson et al., 2004), as well as no responses (Schmidt et al., 2002; Emmett et al., 2004).

Microbial biomass C in the drought plots was higher than in the control plots throughout the whole experiment. Even at the first sampling date (62 days), when the added litter had not experienced a drought treatment yet. A possible explanation could be that when we sampled the litterbags at 62 days, the moisture content of all the litterbags was extremely low. Weather conditions before the sampling date were dry and the litter dried out quickly. It is possible that the colonizing microbial community in the drought plots was more adapted

to these dry conditions (previous drought treatments) and therefore could increase faster. This may indicate a microbial community with a relatively larger contribution of fungi, because fungi are able to extend their hyphae to reach nutrients actively down to matrix potentials at which mobility of bacteria is considered negligible (Griffin, 1981a). The microbial community had a significantly higher C/N ratio in the drought plots as in the control plots at 62 days and 160 days, which suggests that the drought treatment enhanced the fungal dominance of the colonizing microorganisms (Killham, 1994). At 160 days, during peak drought, microbial CO_2 respiration rate was very low (Fig. 2). Nevertheless microbial biomass C in the drought plots was still significantly higher than in the control plots. This signifies that microbes were present in the litter, however their activity was very low. In contrast to microbial biomass C, microbial biomass N and P was considerably smaller at this day (Fig. 3b and c), which is also reflected in a lower total N and P (concentration) (Fig. 4b–d). Low moisture content has more often been found to have a strong effect on microbial biomass N and P (Jensen et al., 2003). Several mechanisms are reported that can cause low microbial biomass at low volumetric soil water content: reduced diffusion of soluble substrates (Griffin, 1981b) and/or reduced microbial mobility and consequent access to substrate (Griffin, 1981a; Killham et al., 1993). In addition, severe drying and rewetting cycles could induce microbial cell lysis (Grierson et al., 1998; Turner et al., 2003). However this third mechanism seems unlikely as released N and P could not be leached from the litterbags due to the drought and would have been measured as (a part of) total N and P. Why microbial biomass C had increased, in spite of low moisture content remains unclear. Although Jensen et al. (2003) also found that summer drought affected microbial biomass N relatively more than microbial biomass C. At 382 days, 7 months after the end of the first drought treatment, microbial activity had fully recovered (Fig. 2). By the drying and rewetting process new substrate may have become available by aggregate disruption, litter fragmentation and substrate desorption and redistribution of water, oxygen, substrate and microorganisms (Lund and Goksøyr, 1980; Sommers et al., 1981; Kieft et al., 1987; van Gestel et al., 1993a,b). It is likely that microorganisms could increase their growth and activity because of this new available substrate and therefore microbial biomass increased. Although microbial biomass C and N is higher in the drought plots at 382 and 628 days, the C/N ratio of the microbial population varied between 6 and 8 and was equal across treatments (Fig. 3d). This suggests that the microbial community in this stage was dominated by bacteria (Ross and Sparling, 1993; Killham, 1994). Total N concentration increased from 0 to 628 days in all plots. This response for N was also found by Tietema (1993) for a litterbag experiment with oak leaves. It signifies a relative slower release of nitrogen compared to carbon, which can be attributed to a relatively large part of nitrogen being bound in slowly decomposing compared to faster decomposing compounds, the importance of N immobilization into microbial biomass and/or the high atmospheric N deposition at the site. Total N concentration in the litterbags in the control plots increased by 4.79 g kg^{-1} between 0 and 628 days. Microbial N immobilization accounted for 1.5 g kg^{-1} of this

increase and atmospheric N deposition accounted for $2.51 \text{ g N (kg litter in litterbag)}^{-1}$ (N deposition of $20 \text{ kg N ha}^{-1} \text{ year}^{-1}$ corresponds to $0.028 \text{ g N litterbag}^{-1}$; average litterbag contents was 0.0124 kg). This means that microbial N immobilization, atmospheric N deposition as well as more C than N loss from the litterbags, must have played a role in the increase in total N concentration. As atmospheric P deposition was very low (Schmidt et al., 2004), total P concentration could increase due to relative more C than P loss and microbial P immobilization (also from outside the litterbag). Considering the high percentage of total P concentration that was immobilized in microbial biomass (81–88%), microbial P immobilization was an important factor causing increased P concentration in the litterbags. The first drought treatment retarded microbial N and P immobilization. In the control and heated plots the period of net N and P immobilization is followed by a period of net mineralization. However, in the drought plots, the net N and P immobilization period was delayed and was not followed by a net N and P mineralization period. The decrease in net N mineralization in litterbags in the heating treatment is in contrast to the increased N leaching beneath the rooting zone (Schmidt et al., 2004). This indicated enhanced N mineralization in the deeper soil horizons and ongoing N saturation of the system. A trend towards decreased foliar P concentrations in the heated plots indicated a relatively larger increase in plant biomass as in P uptake and highlighted a progressive importance of P limitation in restraining plant productivity (Peñuelas et al., 2004). The N leaching in the drought plots remained equally high like in the control plots (Schmidt et al., 2004) and decreased foliar P concentrations also indicated increased P limitation in spite of decreased plant growth in the drought plots (Peñuelas et al., 2004).

One of the characteristics of heathland ecosystems is the adaption of the vegetation to a low availability of nutrients (Aerts and Chapin, 2000). A decrease in N and P availability may decrease grass encroachment into lowland heathlands, because grasses have an advantage over heather under richer nutrient conditions (Heil and Aerts, 1993; Diemont, 1996). Although with current levels of N deposition in the Netherlands, the ecosystem will probably remain N saturated. However at a heathland in Denmark, the same treatments, but especially warming, tended to enhance herbivory (heather beetle, *Lochmaea suturalis*) effects. This could occur either by enhancing insect metabolism (directly by warming and indirectly by decreased plant nutritional value likely necessitating larger consumption) or by growth collapse (drought) (Peñuelas et al., 2004). Increased grazing may promote gap formation, which could offer grasses a chance to grow. Longer-term monitoring of the effect of these treatments is therefore needed to assess their impact on plant productivity and plant species composition.

5. Conclusions

Our results show that litter decomposition rate and microbial nutrient transformations were affected by the treatments. Litter decomposition was temporarily enhanced in response to heating and retarded in response to drought. In both

treatments net N and P mineralization decreased and decreased foliar P concentrations highlighted the importance of P limitation in restraining plant growth in this N saturated ecosystem.

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