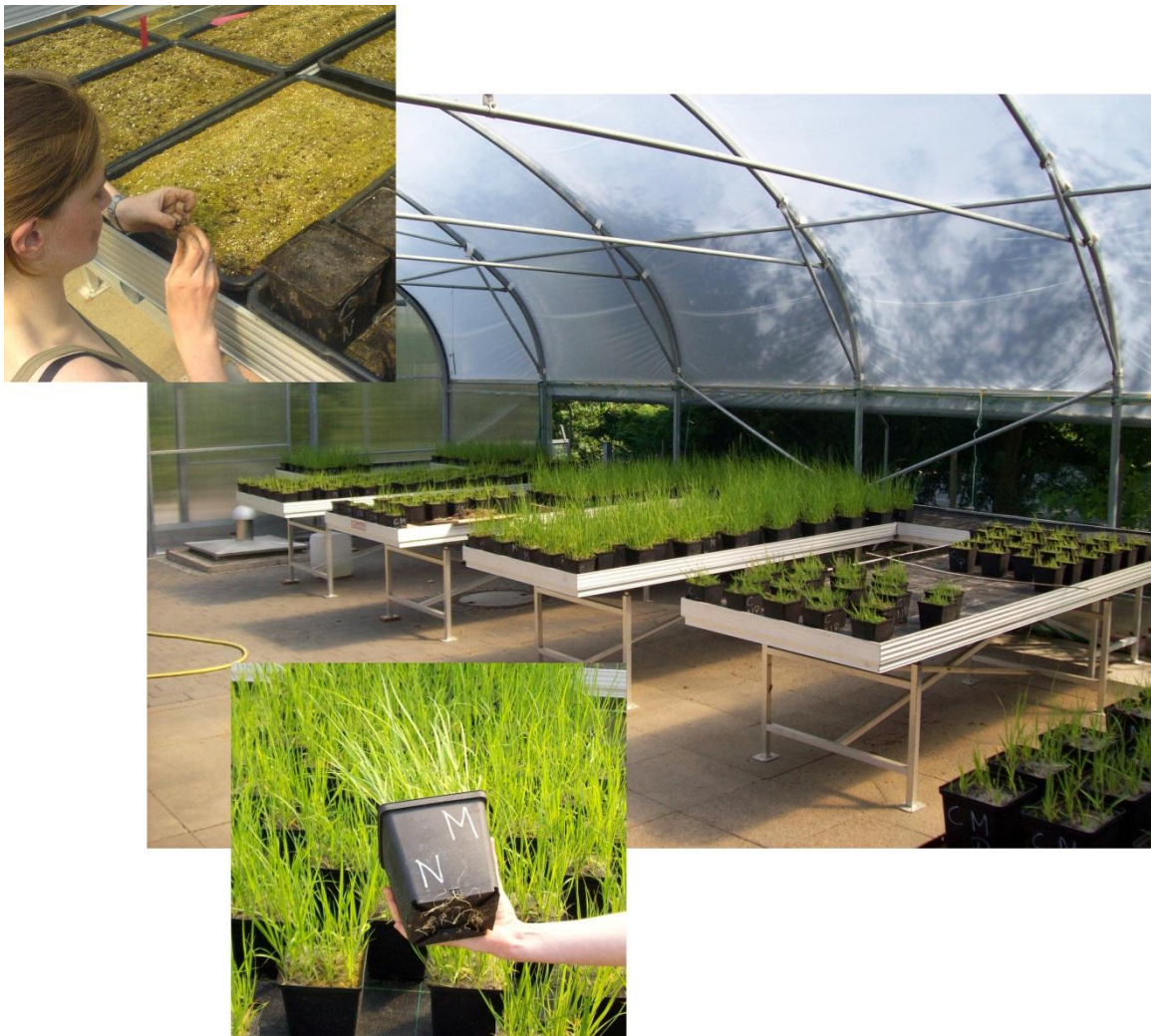


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Mechanisms of purple moor-grass (*Molinia caerulea*) encroachment in dry heathland ecosystems with chronic nitrogen inputs

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Mechanisms of purple moor-grass (*Molinia caerulea*) encroachment in dry heathland ecosystems with chronic nitrogen inputs

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Abstract

We analysed growth strategies (biomass allocation, nutrient sequestration and allocation) of heather (*Calluna vulgaris*) and purple moor-grass (*Molinia caerulea*) seedlings in monocultures and mixtures in relation to N, P, and N+P fertilisation in a greenhouse experiment in order to simulate a heath's pioneer phase under high airborne nitrogen (N) loads. N fertilisation increased the total biomass of both species in monocultures. In mixtures, *M. caerulea* sequestered about 65% of the N applied, while *C. vulgaris* suffered from N shortage (halving of the total biomass). Thus, in mixtures only *M. caerulea* will benefit from airborne N loads, and competition will become increasingly asymmetric with increasing N availability. Our results demonstrate that the heath's pioneer phase is the crucial tipping point at which the competitive vigour of *M. caerulea* (high belowground allocation, efficient use of belowground resources, shortened reproductive cycles) induces a shift to dominance of grasses under increased N availability.

Capsule:

The present study indicates that the heath's pioneer phase is the crucial tipping point at which a shift to dominance of purple moor-grass occurs under increased N availability.

Key-words: biomass allocation, *Calluna vulgaris*, competition, N deposition, nutrient sequestration

Introduction

Airborne N loads are a main driver for biodiversity loss worldwide (Sala et al., 2000). Atmospheric N deposition affects ecosystem nutrient levels and cycles, the growth of plants and their competition, and is responsible for shifts in the species composition of many plant communities (Remke et al., 2009; Friedrich et al., 2011). In heathland ecosystems, N deposition has increased primary productivity and N contents of biomass and soil compartments, and contributed to an increasing susceptibility of dwarf shrubs to secondary stress factors such as frost, drought and heather beetle infestation (Uren et al., 1997; Power et al., 1998). In addition, the replacement of dwarf-shrubs by grasses such as wavy hair-grass (*Deschampsia flexuosa*) and purple moor-grass (*Molinia caerulea*) is seen as a long-term effect driven by ongoing N loads (Heil and Diemont, 1983). Due to the dramatic loss of heathland area Europe-wide caused by a far-reaching structural change in agriculture, heaths are nowadays regarded as an internationally endangered habitat type of high conservation value, and conservation efforts are directed towards the preservation of heathland biodiversity (Webb, 1998).

Encroachment of *M. caerulea* was initially observed in wet heaths and bogs, where cross-leaved heath (*Erica tetralix*) is the dominant dwarf shrub. There is evidence that *M. caerulea* gains a competitive advantage after N fertilisation or after lowering the groundwater level (Berendse and Aerts, 1984). Aerts et al. (1991) showed that *M. caerulea* is able to outcompete *E. tetralix* under high N loads because of its improved productivity, its high belowground biomass allocation and its plasticity in the spatial arrangement of leaves.

In dry heathlands, where heather (*Calluna vulgaris*) is the main competitor of *M. caerulea*, competitive relationships are more complicated, and competition experiments have produced contradictory results (Aerts and Bobbink, 1999; Falk et al., 2010).

In fertilisation experiments conducted by Aerts et al. (1990), *C. vulgaris* proved to be the superior competitor, even at fertiliser additions of 200 kg N ha⁻¹ y⁻¹. Further studies identified fundamental differences between *C. vulgaris* and *M. caerulea* in terms of their nutrient cycling and growth strategies and, thus, their competitive behaviour in dry heaths. *Calluna*

vulgaris, for example, is well adapted to nutrient-poor conditions due to its ericoid mycorrhiza (Read, 1991), its low potential growth rates and a long mean residence time of nutrients in its biomass (Aerts et al., 1991). This “nutrient conserving strategy”, in combination with its evergreen habit, allows *C. vulgaris* to form and sustain a closed canopy all the year round, which in turn may suppress or prevent the establishment of potential competitors, even under increasing nutrient availability (Aerts, 1993a). *Molinia caerulea*, by contrast, is characterised by high potential growth rates, but a short mean residence time of nutrients, which in its turn is accompanied by a higher nutrient demand (Aerts and Berendse, 1988; van Heerwaarden et al., 2005). Because of its deciduous habit, *M. caerulea* has to build up its aboveground biomass at the beginning of each growing season.

If *C. vulgaris* is the superior competitor in dry heaths even under increasing N availability, the question arises as to the nature of the processes underlying the large-scale replacement of *C. vulgaris* by *M. caerulea* observed in many dry lowland heaths of NW Europe during the last two decades (Berendse et al., 1994; Taylor et al., 2001; Falk et al., 2010). Aerts (1993a) contends that *M. caerulea* encroachment in dry heaths occurs when the *C. vulgaris* canopy is opened, for example as a result of senescence, frost, drought, and heather beetle attacks, all of which weaken the competitive power of *C. vulgaris*. This hypothesis is in agreement with the findings of Berendse et al. (1994), according to which the cover of grasses increased after a severe heather beetle attack in NPK-fertilised *C. vulgaris* stands. Bruggink (1993) found that *M. caerulea* seedlings established at dry heath sites where ageing *C. vulgaris* shrubs have been removed by high-intensity management measures such as sod-cutting.

These findings indicate that the dwarf shrub’s rejuvenation phase (i.e. pioneer phase sensu Gimingham, 1972) may be the crucial stage for the establishment of *M. caerulea* in a *C. vulgaris* dominated heath, regardless of whether this has been initiated by natural (e.g. senescence) or man-made disturbance factors (e.g. management). However, studies analysing the competitive relationships between *C. vulgaris* and *M. caerulea* have focused primarily on later successional stages of a heath (e.g. the building or mature phase), and hence paid little attention to the establishment potential of *M. caerulea* in the course of a

heath's pioneer phase. Thus, little is known about the competitive behaviour of *C. vulgaris* and *M. caerulea* seedlings in a heath's pioneer phase, particularly under modifying impacts of airborne N loads. In this context, it is of particular interest to determine whether the species' "growth strategies" (in terms of biomass and nutrient allocation, allometric relationships, and nutrient conserving strategies) that have been analysed with a focus on adult plants also allow a characterisation of the growth behaviour of the species' seedlings. Species-specific growth strategies may change with plant size and thus may be size-dependent (Müller et al., 2000). A plant's growth strategy, however, largely determines its ability to capture resources and to compete with neighbours (Grime, 1979; Tilman, 1988). In a heath's pioneer phase, growth strategies of seedlings of competing species (i.e. *C. vulgaris* vs. *M. caerulea*) may thus determine the course of subsequent successional stages (Gimingham, 1972). *Calluna vulgaris*'s nutrient conserving strategy, for example, should be of minor importance, if seedlings are competing for belowground resources. Adult *M. caerulea* plants, by contrast, are characterised by a high belowground allocation of biomass and nutrients (Aerts et al., 1991). If this also applies to the seedlings, *M. caerulea* should be the superior competitor for belowground resources in a heath's pioneer phase, particularly under elevated N inputs.

The overall objective of the present study is to contribute to a better understanding of the mechanisms underlying the ongoing process of *M. caerulea* encroachment in dry lowland heaths. We hypothesise that the pioneer phase of a heath is the crucial tipping point at which a formerly dwarf shrub dominated heath may become dominated by grasses. We further hypothesise that growth strategies of *C. vulgaris* and *M. caerulea* seedlings are crucial for an understanding of shifts in the species composition (i.e. replacement of *C. vulgaris* by *M. caerulea*), as observed in many European dry lowland heaths.

To this end, we investigated growth strategies of, and competitive relationships between, *C. vulgaris* and *M. caerulea* seedlings in a greenhouse experiment in order to assess their competitive performance by means of a simulated pioneer phase. We focused on growth variables such as biomass and nutrient allocation patterns of both species in monocultures

and mixtures, and analysed shifts in their competitive behaviour as affected by N, P, and N+P fertilisation. The following questions were addressed: (i) What are the main mechanisms (in terms of growth strategies such as biomass allocation and nutrient sequestration) that control the competitive performance of *C. vulgaris* and *M. caerulea* seedlings in a heath's pioneer phase? (ii) How does N and P availability affect the competitive performance of both species in this phase? (iii) Do seedlings and adults of *C. vulgaris* and *M. caerulea* differ in their growth strategies?

Material and methods

Experimental design

Calluna vulgaris and *M. caerulea* seeds were collected in September 2007 in the Lüneburger Heide nature reserve and transferred to germination dishes in a greenhouse without temperature control. Seedlings emerged in the middle of March 2008 (*M. caerulea*) and at the beginning of April 2008 (*C. vulgaris*). For the competition experiment we prepared plant pots (12 x 12 x 12 cm³) that contained nutrient-poor sand (7 cm thick), covered by a humus layer (4 cm thick). The sand was taken from a local sand pit (i.e. C-material typical of podzols; soil ecological characteristics: pH_{H2O}: 7.1, base saturation: 100%, S-value: 4.8 mval 100 g⁻¹, N content: 100 mg kg⁻¹, P content: 95 mg kg⁻¹). The humus material was collected from the upper soil horizons in the Lüneburger Heide nature reserve (soil ecological characteristics: pH_{H2O}: 3.8, base saturation: 21.5%, S-value: 1.7 mval 100 g⁻¹, N content: 800 mg kg⁻¹, P content: 147 mg kg⁻¹). A total of 16 individuals were planted in each pot (4 × 4 rows) in three planting schemes (i.e. competition types): (i) *C. vulgaris* monocultures (i.e. 16 individuals of *C. vulgaris*; henceforth referred to as C-pots); (ii) *M. caerulea* monocultures (i.e. 16 individuals of *M. caerulea*; henceforth referred to as M-pots), and (iii) *M. caerulea*-*C. vulgaris* mixtures (i.e. 8 individuals of *C. vulgaris* and *M. caerulea* respectively; henceforth referred to as CM-pots). This planting scheme was used to mimic natural rejuvenation patterns in a heath's pioneer phase and corresponded to seedling densities characteristic of dry lowland heaths of the Lüneburger Heide nature reserve (Fottner et al., 2004). A total of 40 pots per competition type were set up (i.e. total n of pots was 120). 10 pots per competition type were randomly assigned to four different nutrient treatments: control, N, P, and N+P (i.e. 10 replicates per competition type and nutrient treatment). N-treated pots received 48 kg N ha⁻¹ y⁻¹ (NH₄NO₃), P-treated pots received 4 kg P ha⁻¹ y⁻¹ (Na₂HPO₄) and N+P treated pots received a combination of both. Control pots received deionised water only. Nutrient solutions were applied weekly from the end of May until the end of August. Pots were kept in a greenhouse without temperature control and were watered regularly to

prevent water stress. The pots in the greenhouse were re-arranged randomly every four weeks.

Harvesting and chemical analyses

Plants from all pots were harvested at the beginning of September (when plants were still in a fresh and green state). For the analyses of growth responses to fertilisation we focused on the morphological traits that are considered good indicators of the competitive performance of the respective species (Tomassen et al., 2003; Tomassen et al., 2004). Accordingly, we quantified the aboveground and belowground biomass production of both species in the different competition types and nutrient treatments (by analysing all plant individuals of the pots). In addition, we determined the percentage of flowering plants per pot, the number of tillers per plant (only for *M. caerulea*), the shoot-root ratios, and N and P biomass concentrations (to assess nutrient sequestration and allocation patterns).

After harvest, biomass samples were dried at 80°C for 12 h (until weight constancy) and weighed. Subsequently, samples were sheared with an ultra centrifugal mill (ZM 200, Retsch, Haan, Germany), ground with a mixer mill (MM 400, Retsch, Haan, Germany), and re-dried over night at 105 °C. N contents were analysed with a C/N analyser (Vario EL cube; Elementar, Hanau, Germany). For P determination, samples were incinerated in a muffle furnace (N7; Nabertherm, Lilienthal, Germany) at 550° C for three hours. Ash samples were dissolved in an HCl solution, which was evaporated to dryness. Residues were again dissolved in an HCl solution (Schlichting et al., 1995). Analyses were performed using an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES; Optima 3300 RL; Perkin Elmer, Burladingen, Germany).

Data evaluation and statistics

Treatment effects on pot means of biomass dry weights, shoot-root ratios, tissue N and P concentrations and the number of tillers (only *M. caerulea*) were compared by means of one-way ANOVA with a Tukey's post hoc test ($P < 0.05$). Interrelated treatment effects (i.e. type

of fertilisation, competition type) were tested using Generalized Linear Models (GLM). Each GLM included the main effects of N, P, and competition type (henceforth referred to as CT: monoculture vs. mixture) as well as the first order interaction terms (N x P, N x CT, P x CT; see Fig. 1 and Table 1; all data refer to plant individuals of *C. vulgaris* and *M. caerulea*).

In order to evaluate the plants' N and P sequestration, total biomass N and P contents (product of the total biomass and the tissue N and P concentrations) were calculated per pot and species (Fig. 2). To quantify the plants' fertiliser uptake, the total biomass N and P contents of controls were subtracted from the total biomass N and P contents of corresponding treatments. Data in Fig. 2 are presented as percentage uptake from applied quantities of fertiliser per pot and species. One-way ANOVA and Tukey's post hoc test were performed to compare mean fertiliser uptake rates per pot and competition type. Inter- and intraspecific competition was assessed by means of replacement diagrams (Jolliffe, 2000).

All analyses were conducted with SPSS 17.0 (SPSS Inc., Chicago, IL). Non-normally distributed data (percentage of flowering plants per pot) were subjected to Kruskal-Wallis-H-Test with multiple comparisons ($P < 0.05$; Bortz et al., 2008; using STATISTICA 7.1 (StatSoft Inc., Tulsa, OK)).

Results

Treatment effects on C. vulgaris

In the C-pots, aboveground biomass increased by about 60% and 240% as a result of N and N+P fertilisation, respectively (Fig. 1a). Effects of the P treatment were insignificant, but we found a significant N x P interaction on aboveground biomass productivity (Table 1). Belowground biomass decreased as a result of the N treatment (corresponding with a reduction in the relative belowground biomass allocation from 38% to 19%; calculated from data in Table 1), but did not change significantly under N+P fertilisation (Fig. 1b). Again, there was no significant P effect. Shoot-root ratios were highest in the N and N+P treatments due to both a decrease in the belowground (N treatment) and an increase in the aboveground biomass (N and N+P treatments; Fig. 1c). The percentage of flowering plants was only affected in the N+P treatment, and increased from 2% in the control to 48% in N+P treated pots (Fig. 1d).

In the CM-pots, aboveground productivity was distinctly low in all treatments, and productivity was even lower in the N and N+P treatments than in the controls of the C-pots (Fig. 1a). The same response pattern was found for the belowground biomass, and only the N+P treatment enhanced belowground productivity (Fig. 1b). Treatments did not affect the shoot-root ratios in the CM-pots (Fig. 1c). In addition, *C. vulgaris* was almost flowerless in the presence of *M. caerulea* (Fig. 1d).

N and N+P treatments approximately doubled tissue N concentrations of *C. vulgaris* in the C-pots, but effects of these treatments were distinctly reduced in the CM-pots (Table 1). In contrast, P and N+P treatments increased tissue P concentrations in the C-pots. In addition, we found a significant N x P interaction effect on tissue N concentrations.

In the C-pots, *C. vulgaris* sequestered 19% and 43% of experimentally added N in the N and the N+P treatment, respectively (Fig. 2a). P sequestration was 1% in the P and 38% in the N+P treatments. In contrast to the C-pots, N and P uptake of *C. vulgaris* dropped distinctly in the presence of *M. caerulea*, and uptake rates were less than 2% for N and P.

Treatment effects on M. caerulea

In the M-pots, aboveground biomass was more than sixfold higher in the N and fivefold higher in the N+P treatments (Fig. 1e), and N x P interactions on aboveground biomass were significant (Table 1). Belowground biomass quadrupled as a result of N and tripled as a result of N+P fertilisation (Fig. 1f). P treatments affected neither aboveground nor belowground productivity. Both the shoot-root ratios and the number of tillers were twice as high in the N and N+P treatments than in the control pots (Fig. 1g,h), and flowering plants were only developed in the N and N+P treatments (Fig. 1i).

Aboveground and belowground productivity of *M. caerulea* achieved maximum values in the CM-pots subjected to N and N+P fertilisation (Fig. 1e,f), and shoot-root ratios doubled in comparison to controls (Fig. 1g). Similarly, the number of tillers and the percentage of flowering tillers were highest in these treatments (Fig. 1h,i).

N and N+P treatments only slightly affected tissue N concentrations of *M. caerulea* (in both the M- and CM-pots; Table 1). P concentrations were increased as a result of P fertilisation (both competition types), but remained unchanged (M-pots) or decreased (CM-pots) in the N+P treatments. N x P interactions on tissue N and P concentrations were insignificant (Table 1).

In the M-pots *M. caerulea* sequestered 54% (N+P treatment) and 63% (N treatment) of experimentally added N, and uptake rates were hardly affected by the presence of *C. vulgaris* (Fig. 2c). P uptake was highest in N+P treatments (Fig. 2d, both competition types). Calculation of P uptake in P treated CM-pots resulted in a negative value, indicating that P uptake in non-fertilised controls was higher than in P treated pots.

Treatment effects on the competitive performance of C. vulgaris and M. caerulea

Treatment effects on the competitive performance of *C. vulgaris* and *M. caerulea* were assessed by means of replacement diagrams (Fig. 3). Results showed that intraspecific competition was weaker than interspecific competition for *C. vulgaris* (as indicated by a concave curve progression), but was stronger for *M. caerulea* (as indicated by a convex

curve progression). In the N and N+P treatments, the convex shape of the *M. caerulea* curves became more marked (Fig. 3b,d vs. Fig. 3a), indicating an increasing competitive advantage of *M. caerulea* with increasing N supply. In contrast to N and N+P treatments, productivity patterns for both species in the P treatment did not differ from those in the controls (Fig. 3c vs. Fig. 3a).

Discussion

Treatment effects on C. vulgaris monocultures

Responses of *C. vulgaris* to nutrient treatments indicated that growth was NP co-limited, whereby N was the principally limiting nutrient (Table 1, Fig. 1). This was reflected by significant increases in the plants' productivity following N fertilisation, but by maximum biomass values in the N+P treatments (Table 1). In addition, N+P treatments significantly improved the floral development of plants (Fig. 1d). Positive responses of *C. vulgaris* to N fertilisation have also been found in other field or greenhouse experiments (Heil and Bruggink, 1987; Aerts et al., 1991, Uren et al., 1997; Power et al., 1998), although full-factorial additions of N and P have been applied only once in a field study of von Oheimb et al. (2010). A shift from N to P or NP co-limitation may take place when two nutrients (i.e. N and P) are potentially limiting for plant growth, but when fertilisation with N will cause P to become relatively scarce ("resource-ratio hypothesis", Tilman, 1985). In heaths such a shift has been observed as a result of chronic inputs of N from the atmosphere (Verhoeven et al., 1996; Roem et al., 2002). Strikingly, N treatments halved the relative belowground biomass allocation of *C. vulgaris* seedlings (from 38% to 19%), accompanied by a distinct increase of shoot-root ratios (controls: 1.6; N treatment: 4.3; Table 1; indicating a different growth behaviour of seedlings compared to that of the 2-4 year old plants; cf Aerts et al. (1991).

Treatment effects on M. caerulea monocultures

In contrast to *C. vulgaris*, *M. caerulea* was exclusively limited by N. Aboveground and belowground productivity was highest in the N treatment, and N fertilisation doubled the number of tillers per seedling (Table 1). This is in agreement with results found for older plants (Thornton, 1991; Güsewell et al., 2003; van Heerwaarden et al., 2005) according to which *M. caerulea* is characterised by high demands for N, but low P requirements (Roem et al., 2002). In addition, "secondary tillering" as a result of improved N supply seems to be a characteristic growth pattern of *M. caerulea* (Thornton, 1991). In contrast to the study by Heil and Bruggink (1987), *M. caerulea* growth was not promoted in the P treatment, and even

inhibited in the N+P treatment in comparison to the N treatment (Fig. 1e). It is, thus, likely that additional factors such as water supply or mycorrhizal infection may mediate outcomes of fertilisation experiments (Aerts and Bobbink, 1999; Falk et al., 2010). Decreasing growth responses in the N+P treatment may be attributable to ion competition between NO_3^- and PO_4^{3-} , resulting in a decreasing N uptake in the N+P fertilised pots (Fitter and Hay, 2002). As indicated by low shoot-root ratios (ranging between 0.5 and 1.0), *M. caerulea* seedlings showed a high percentage biomass allocation to the roots, even in the N treatment. Seedlings thus exhibit allocation patterns that are well comparable to older plants (shoot-root ratios of 2-4 year old *M. caerulea* showed the same range in the study by Aerts et al. (1991)). These findings have important implications for the sequestration of nutrients and, thus, the competitive performance of *M. caerulea* seedlings (see discussion in the following paragraph).

Treatment effects on competitive interactions between C. vulgaris and M. caerulea

In the CM-pots *M. caerulea* was clearly the superior competitor to *C. vulgaris*. This was attributable to the fact that *M. caerulea* seedlings have the potential to utilise an improved N availability to multiply their biomass production. N fertilisation, for example, increased total biomass of *C. vulgaris* seedlings by a factor of 1.2, but total biomass of *M. caerulea* seedlings by 4.8 (Table 1). This positive response pattern of *M. caerulea* to increasing N availability was particularly pronounced in the CM-pots, expressed by a ninefold increase in the total biomass of *M. caerulea* seedlings (in comparison to the control M-pots), accompanied by a halving of the total biomass of *C. vulgaris* seedlings (in comparison to the control C-pots). *Molinia caerulea* seedlings thus exhibited a much stronger response to N fertilisation than that known from older plants (cf. Aerts et al., 1991)

Based on our findings, we hypothesise that the competitive superiority of *M. caerulea* over *C. vulgaris* is linked to the following (partly interrelated) mechanisms, all of which will favour *M. caerulea* encroachment in a heath's pioneer phase under high N loads:

(i) The high belowground biomass allocation of *M. caerulea* seedlings; (ii) A decisive advantage of *M. caerulea* seedlings in the competition for belowground resources (i.e. N); (iii) A (subsequently) high aboveground productivity; (iv) Shortened reproductive cycles of *M. caerulea* (i.e. after one year) in high N environments; (v) NP co-limitation of *C. vulgaris* seedlings (instead of a sole N limitation of *M. caerulea* seedlings); (vi) A reduced relative belowground allocation of *C. vulgaris* seedlings following N fertilisation.

(i) *Molinia caerulea* seedlings showed a distinctly higher belowground biomass allocation than *C. vulgaris* seedlings. This was mirrored by the very low shoot-root ratios found for *M. caerulea* (0.5 and 1.0 in the controls and N treatments of the M-pots, respectively; Table 1). *Calluna vulgaris*, in contrast, achieved shoot-root ratios of 1.6 and 4.3 (controls and N treatments of C-pots, respectively). As a consequence of a high belowground investment, *M. caerulea* seedlings suppressed the root development of *C. vulgaris* seedlings in all treatments in the CM-pots, as reflected by a reduced *C. vulgaris* belowground biomass and unchanged shoot-root ratios for *M. caerulea*.

(ii) The high belowground biomass allocation of *M. caerulea* seedlings explains their competitive superiority for belowground resources (Goldberg, 1990). An impressive finding was that N sequestration of *M. caerulea* seedlings was unaffected by the presence of *C. vulgaris*, since N uptake rates of *M. caerulea* in the N treatment were almost the same in the M- and CM-pots (about 65%; Fig. 2c). N sequestration of *C. vulgaris*, in contrast, showed a distinct decrease as a result of interspecific belowground competition (Fig. 2a), although *C. vulgaris* may partly compensate a lower belowground allocation by a higher specific root length as well as by its ericoid mycorrhizas (Boot, 1989; Read, 1991, Aerts, 1993b). In our experiments, the high N uptake of *M. caerulea* hardly affected the plants' tissue N concentrations (Table 1). This is indicative of the high N demands of *M. caerulea* seedlings and the use of sequestered N to increase the plants' biomass production (Aerts and Berendse, 1988; van Heerwaarden et al., 2005). By contrast, low tissue N concentrations of *C. vulgaris* seedlings (in the CM-pots) showed that growth was limited by N in the entire course of the experiment. This interpretation is supported by the finding that productivity of *C.*

vulgaris in mixtures did not differ for N and N+P treatments, because the plants' N demand was not satisfied in competition with *M. caerulea* (Fig. 1a,b).

For our experiment we rule out that mycorrhizal infection rates of plant roots in the pots significantly differed from those under field conditions, which in turn might have affected experimental outcomes. There is evidence from both pot and field experiments that mycorrhizal infection takes place in parallel with the development of the fine roots of the plants (immediately after their development; Fyson and Oaks, 1992; Smith, 2009). Since the infection rate of roots follows a logistic growth curve, high infection rates are achieved within a few weeks after seedling growth (Fyson and Oaks, 1992; Smith, 2009).

(iii) As a consequence of the superior competition for belowground resources, *M. caerulea* seedlings multiplied their aboveground productivity in the N and N+P treated pots. Seedlings thus responded even more strongly to N fertilisation than older plants (cf. experiments by Aerts et al., 1991). As shown in Fig. 3, N fertilisation strengthened the intraspecific competition among *M. caerulea* seedlings, and their biomass almost doubled in the CM-pots as a result of reduced intraspecific competition (compared to the M-pots under N fertilisation; Table 1). *Molinia caerulea* seedlings clearly optimise their productivity (and their investment in reproductive tissue; see following paragraph) by an optimal exploitation of plant-available N resources, and *C. vulgaris* seedlings were unable to interfere with this process. Although *M. caerulea* seedlings proved to be strong competitors to *C. vulgaris* seedlings even in unfertilised pots, our results demonstrated that only *M. caerulea* seedlings will benefit from increasing N availability. Thus, competition will become increasingly asymmetric with increasing N inputs. In contrast, growth strategies that are typical of many *Ericaceae* adapted to low N environments (such as low potential growth rates, production of long-living tissue), prove to be a competitive disadvantage when N availability increases (Aerts, 1990; Aerts et al., 1991; Aerts, 1993b). The nutrient-conserving strategy and the evergreen habit of *C. vulgaris* may explain its competitive vigour in closed stands, for example in the late building phase of a heath when *C. vulgaris* suppresses *M. caerulea* encroachment even under high N

loads (Aerts, 1993a). In the pioneer phase of a heath, however, when seedlings compete for belowground resources, these traits are less effective, particularly in high N environments.

(iv) *Molinia caerulea* was capable of shortening its reproductive cycle to one year, as indicated by a high percentage of fertile plants in the N treatments (Fig. 1i). Since *M. caerulea* may also shift from an N recycling to a reserve formation strategy under high N conditions (van Heerwaarden et al., 2005), plants tend to develop a higher proportion of generative tillers (Aerts and de Caluwe, 1989; Falk et al., 2010). Both strategies increase the number of seedlings in the pioneer phase and hence accelerate *M. caerulea* encroachment in places where the dwarf shrub canopy has been opened (Aerts and Heil, 1993).

(v) *Calluna vulgaris* seedlings proved to be NP co-limited, while *M. caerulea* seedlings were exclusively limited by N. Since current deposition rates are low for P, but high for N (Härdtle et al., 2006), they disproportionately support the competitive vigour of *M. caerulea* plants. This applies to both the biomass increment rates and the investment in reproductive tissue (i.e. seed formation).

(vi) In contrast to growth strategies that are known from older plants (cf. Aerts et al., 1991), *C. vulgaris* seedlings halved their relative belowground allocation as a result of increasing N availability. This growth behaviour (resulting in shoot-root ratios > 4) may increase the seedlings' susceptibility to drought events, and thus mortality rates during periods of drought (Power et al., 1998). High N inputs may already have weakened the rejuvenation of *C. vulgaris* in the last decades by this mechanism. Since European heaths will face both high inputs of N and shifting climatic conditions in the near future (Galloway et al., 2004; IPCC, 2007), increasing seedling mortality may contribute to a deterioration of *C. vulgaris*'s rejuvenation success in European heathlands.

Conclusions

Our study demonstrated that the pioneer phase of a heath is the crucial tipping point in a heath's succession, in which *M. caerulea* establishment may induce a transition from a formerly dwarf shrub dominated heath to grass dominated swards. Important traits for the

competitive superiority of *M. caerulea* seedlings in the pioneer phase of a heath are high biomass allocation to the roots, an efficient use of belowground resources (in terms of high N sequestration) and, hence, high aboveground productivity (which in turn improves the plants' competitive vigour for light). In addition, N loads shorten the plants' reproductive cycle, accelerating seed production, which then starts in the pioneer phase of a heath. *Calluna vulgaris* seedlings, by contrast, may suffer from the combined effects of high N loads and increasing drought events due to their low belowground allocation (and thus high shoot-root ratios) in high N environments.

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Table 1. Means and standard errors (in parentheses) of aboveground, belowground and total biomass (in mg dryweight (dw)), shoot-root ratios, tissue N and P concentrations (in mg g⁻¹ dw) per plant individual of *C. vulgaris* and *M. caerulea*, and number of tillers (only *M. caerulea*) under the different fertilisation treatments (C: control; N: nitrogen; P: phosphorus; N+P: nitrogen and phosphorus) in monocultures and in mixtures. Results of GLM analyses with interactions of nitrogen (N), phosphorus (P) and competition type (CT; i.e. monoculture vs. mixture) are given where significant: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

<i>C. vulgaris</i>	<i>C. vulgaris</i> monocultures				<i>C. vulgaris</i> - <i>M. caerulea</i> mixtures				Interactions
	C	N	P	N+P	C	N	P	N+P	
Biomass	32.5	52.2	28.0	109.7	8.9	16.1	10.2	17.7	N x P**
above (mg)	(3.1)	(4.7)	(1.6)	(8.2)	(0.8)	(0.9)	(0.6)	(2.1)	N x CT**
Biomass	20.2	12.4	18.8	28.1	4.7	7.1	5.6	8.3	N x P**
below (mg)	(1.4)	(1.1)	(0.9)	(2.8)	(0.4)	(0.8)	(0.7)	(1.3)	N x CT**
Biomass	52.7	64.6	46.8	137.9	13.6	23.2	15.9	26.0	N x P**
total (mg)	(4.3)	(5.5)	(2.3)	(10.4)	(1.1)	(1.7)	(1.2)	(3.4)	
Shoot-root	1.6	4.3	1.5	4.1	2.0	2.4	2.1	2.2	N x CT***
ratio	(0.1)	(0.3)	(0.1)	(0.3)	(0.2)	(0.2)	(0.3)	(0.1)	
N _{conc}	10.0	20.8	10.6	17.7	12.0	13.8	13.5	13.0	N x P***
(mg g ⁻¹)	(0.2)	(0.6)	(0.2)	(0.7)	(0.3)	(0.5)	(0.3)	(0.3)	N x CT*** P x CT*
P _{conc}	1.0	1.0	1.2	1.4	1.6	1.2	1.7	1.3	N x CT***
(mg g ⁻¹)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)	P x CT***
<i>M. caerulea</i>	<i>M. caerulea</i> monocultures				<i>C. vulgaris</i> - <i>M. caerulea</i> mixtures				Interactions
	C	N	P	N+P	C	N	P	N+P	
Biomass	27.4	177.1	24.9	145.6	70.8	334.4	59.1	284.6	N x P***
above (mg)	(1.2)	(4.5)	(1.2)	(6.0)	(4.9)	(8.9)	(4.0)	(12.3)	N x CT***
Biomass	50.7	190.6	43.3	143.8	142.0	334.2	107.6	288.1	N x CT**
below (mg)	(2.6)	(11.2)	(3.3)	(10.1)	(11.1)	(15.0)	(9.0)	(13.8)	
Biomass	78.1	374.2	68.2	289.4	212.8	668.5	166.7	572.7	
total (mg)	(3.6)	(14.8)	(4.5)	(15.5)	(15.3)	(18.0)	(12.8)	(20.5)	
Shoot-root	0.5	1.0	0.6	1.0	0.5	1.0	0.6	1.0	
ratio	(0.0)	(0.1)	(0.0)	(0.0)	(0.0)	(0.1)	(0.0)	(0.1)	
Tillers	1.5	3.1	1.4	3.0	2.4	4.0	2.2	3.9	
	(0.0)	(0.1)	(0.1)	(0.1)	(0.1)	(0.1)	(0.2)	(0.2)	
N _{conc}	6.4	8.6	7.1	9.9	6.3	10.3	7.1	9.6	P x CT*
(mg g ⁻¹)	(0.2)	(0.2)	(0.2)	(0.4)	(0.2)	(0.4)	(0.2)	(0.2)	
P _{conc}	1.0	0.6	1.7	1.0	1.4	0.7	1.6	0.8	P x CT***
(mg g ⁻¹)	(0.1)	(0.0)	(0.1)	(0.1)	(0.1)	(0.0)	(0.1)	(0.0)	

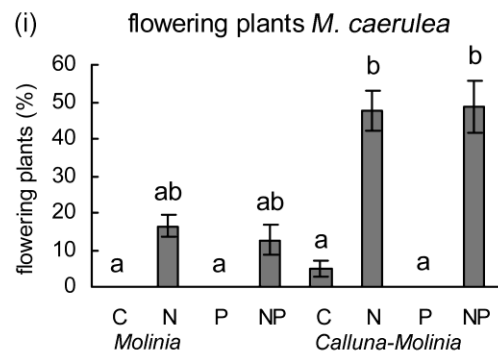
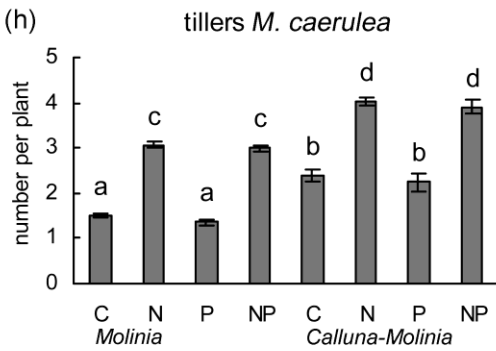
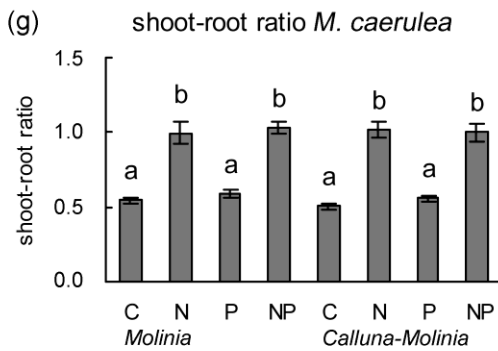
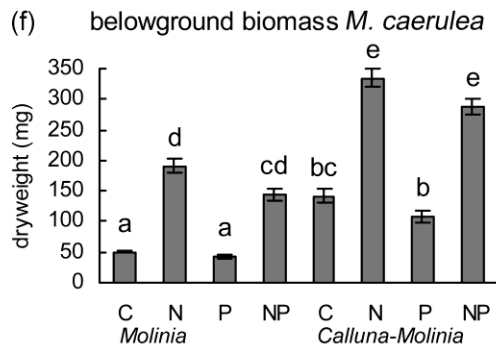
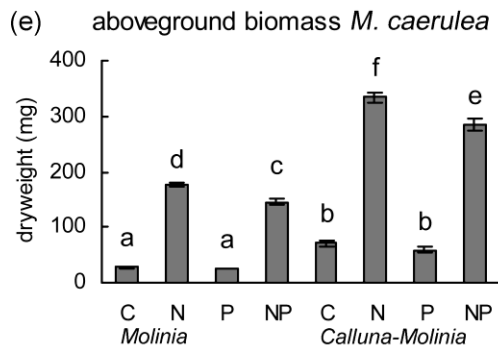
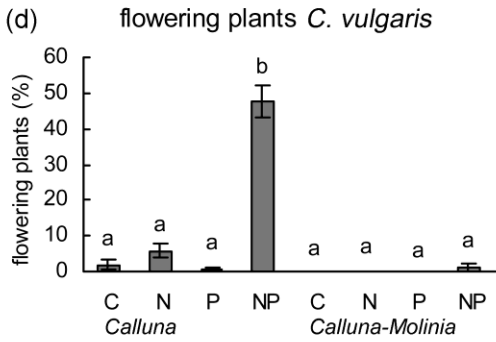
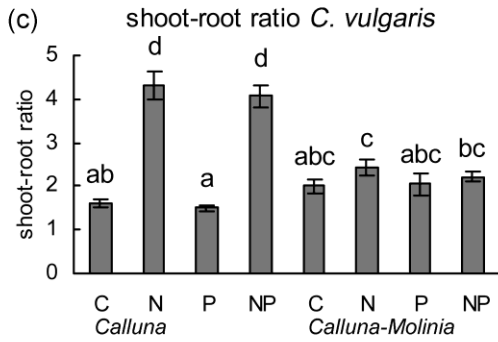
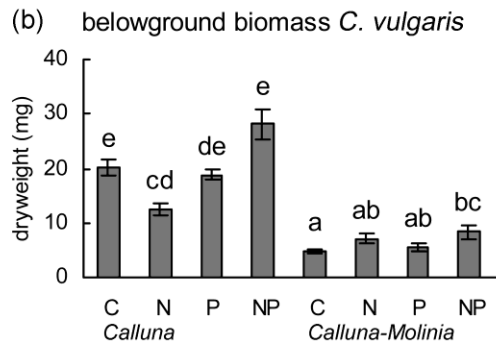
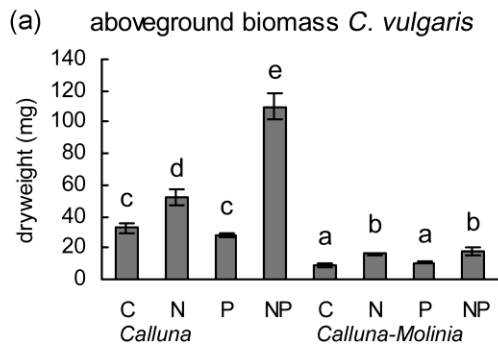


Fig. 1. Effects of fertilisation treatments (C: control; N: nitrogen; P: phosphorus; NP: nitrogen and phosphorus) and competition type (monocultures vs. mixtures) on *C. vulgaris* (*Calluna*) and *M. caerulea* seedlings (*Molinia*). Data refer to plant individuals and show mean and ± 1 standard error (error bars) of: aboveground biomass (a, e), belowground biomass (b, f), shoot-root ratios (c, g), and percentage of flowering plants (d, i) of *C. vulgaris* and *M. caerulea*, respectively. Number of tillers of *M. caerulea* is presented in Fig. 1h. Different letters indicate significant differences ($P < 0.05$).

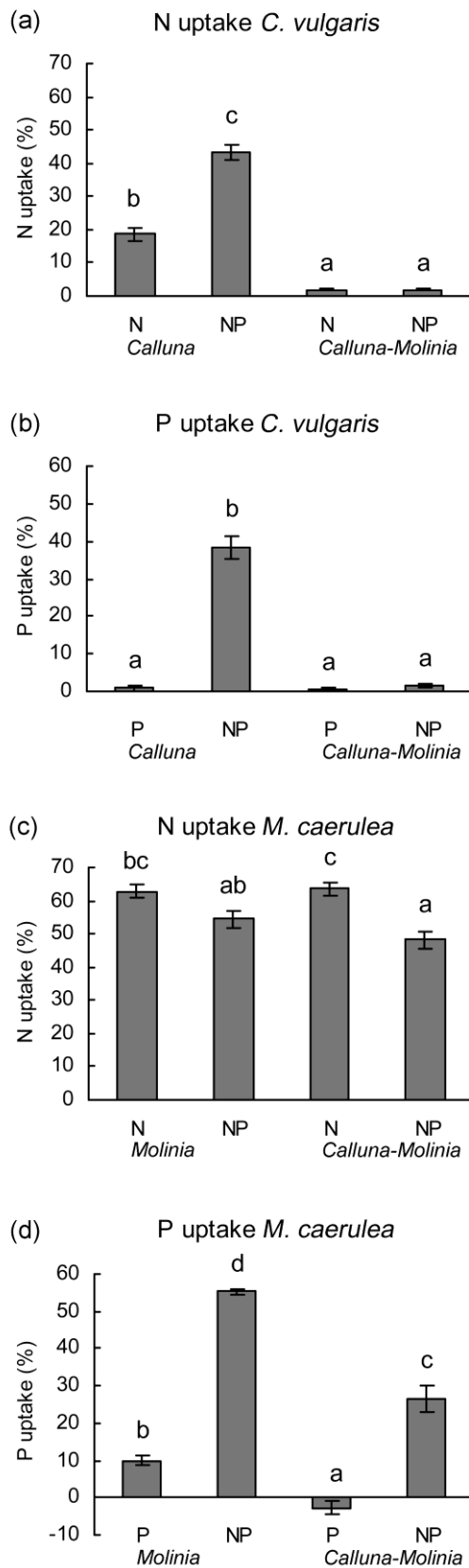


Fig. 2. Mean and ± 1 standard error (error bars) of N (a, c) and P (b, d) fertiliser uptake (%) of *C. vulgaris* (*Calluna*) and *M. caerulea* (*Molinia*), respectively. Data represent total fertiliser uptake per species and pot (i.e. of 16 plant individuals in monocultures and 8 plant

individuals in mixtures). Different letters indicate significant differences ($P < 0.05$) according to one-way ANOVA. Treatments: N: nitrogen; P: phosphorus; NP: nitrogen and phosphorus.

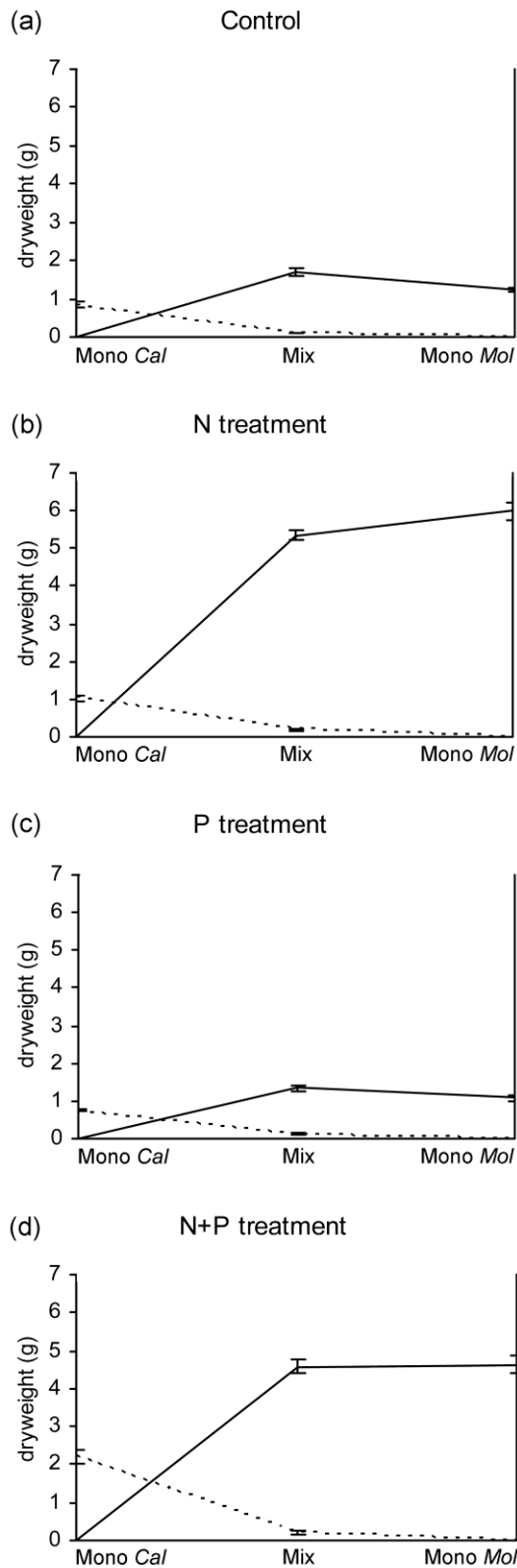


Fig. 3. Replacement diagrams based on mean biomass yields per species and pot (g dw; error bars: ± 1 standard error) for the different fertilisation treatments: control (a), nitrogen (b), phosphorus (c), nitrogen and phosphorus (d). Yields of *C. vulgaris* (dotted lines) represent the biomass of 16 plants in *C. vulgaris* monocultures (Mono Cal), 8 plants in *C. vulgaris* - *M.*

caerulea mixtures (Mix) and 0 plants in *M. caerulea* monocultures (Mono *Mo*). Yields of *M. caerulea* (solid lines) are presented in the same way.

