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Title: **Biomass production, symbiotic nitrogen fixation and inorganic N use in dual and tri-component annual intercrops.**

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5 Abstract

The interspecific complementary and competitive interactions between pea (*Pisum sativum* L.), barley (*Hordenum vulgare* L.) and rape (*Brassica napus* L.), grown as dual and tri-component intercrops were assessed in a field study in Denmark. Focus was on total biomass production and N use at two levels of N fertilisation (0.5 and 4.0 g N / m²), measured at 5 harvest throughout a growing season. All intercrops displayed land equivalent ratio values close to or exceeding unity, indicating complementary use of growth resources. Whereas both rape and barley responded positively to increased N fertilisation, irrespective of whether they were grown as sole- or intercrops, pea was strongly suppressed when grown in intercrop. A suppression of pea that, in both the pea-barley and pea-rape intercrops, lead to a decrease in total uptake of N, most likely resulting from reduced N₂ fixation. Of the three crops barley was clearly the strongest competitor for both soil and fertilizer N, rape intermediate and pea the weakest. Faster initial growth of barley than both pea and rape gave barley an initial competitive advantage, an advantage that in the two dual intercrops was strengthened by the addition of N. Apparently the competitive superiority of barley was less strong in the tri-component intercrop, indicating that the impact of the dominant may have been diminished through indirect facilitation. Interspecific competition had a promoting effect on N₂ fixation of pea, and most so at low N fertilisation. Results indicate that the benefits achieved from the association of a legume

and nonlegume, in terms of N_2 fixed were greatest when pea was grown in association with rape as opposed to barley which could indicate that the benefits achieved from the association of a legume and nonlegume are partly lost if the nonlegume is too strong a competitor.

Introduction

Knowledge of how crop species diversity affects biomass production nutrient cycling and use under temperate cropping conditions is relatively limited and the study of multi-species
5 crops rarely moves beyond two component intercrops. A few studies have addressed these questions in natural ecosystems (Naeem *et al.* 1996; Tilman and Downing 1994; Tilman *et al.* 1996). Increased diversity has been hypothesized to affect cropsystem functions through partitioning of resources (Trenbath 1974; Vandermeer 1990), whereby crops in more diverse communities may increase total resource capture, and thus increase net
10 biomass production. Such complementary resource use could occur in space, in time or in types of resources used (Fukai and Trenbath 1993; Midmore 1993). Species that are deeply rooted have access to water and nutrients not available to more shallowly rooted species (Hauggaard-Nielsen *et al.* 2001b). Differences in shoot architecture may allow intercrops attain a more complete canopy cover of the soil, thereby increasing the overall leaf-area
15 index and light interception of the crop (Keating and Carberry 1993; Vandermeer 1990). Phenological differences may allow crops to utilise resources at different times in the growth season (Fukai and Trenbath 1993; Willey *et al.* 1983). Different species may also use different nutrient sources, such as legumes that can fix atmospheric nitrogen (Anil *et al.* 1998; Carruthers *et al.* 2000; Hauggaard-Nielsen *et al.* 2001a; Jensen 1996) or plants
20 with mycorrhizal mutualisms that allow greater access to organically bound phosphorous (Chiariello *et al.* 1982; Johansen and Jensen 1996). These examples illustrate the potential of complementary resource use by intercrops, however plants also compete strongly for some resources (Tilman 1988; Vandermeer 1989) wherefore optimising intercrop

advantage is achieved by maximizing complementarity and minimising competition between component crops (Vandermeer 1989; Willey 1979).

Many intercrop studies have dealt with the association of two annual crops and the degree
5 of complementarity achieved when two crops are intersown as opposed to sole cropped
(Hauggaard-Nielsen *et al.* 2001a; Jensen 1996; Li *et al.* 1999; Ofori and Stern 1987). The
resulting reduction in competition has been suggested to be the primary reason for
improved yields through intercropping (Vandermeer 1990). Apart from complementary
resource use, facilitation has been suggested as a mechanism of obtaining greater yields in
10 intercrops opposed to sole crops. Facilitation is the mechanism by which some plant
species may have a positive impact on the performance of others. Such beneficial
interactions could be the result of increased resource availability through root induced
changes in the rhizosphere (Ae *et al.* 1990; Horst and Waschkies 1987; Marschner *et al.*
1986; Vandermeer 1990), increased standing ability brought about by the physical support
15 provided by one species to the other, reduced weed pressure through shading or
allelopathic influence (Midmore 1993), reduced pest attack and pathogen infection through
greater biological control in intercrops (Mitchell *et al.* 2002; Trenbath 1993) or as a result
of the resource concentration mechanism whereby host plants, due to greater spacing and
natural barriers formed by other component plants, are harder to find in an intercrop
20 (Trenbath 1993; Vandermeer 1989).

The question of how resource availability affects the relationship between diversity of an
intercrop and its biomass productivity is relatively undebated in the intercrop literature.

Much experimental work has dealt with the impact of nitrogen availability on the

complementarity and productivity of two component cereal-legume intercrops (Ghanbari-Bonjar and Lee 2002; Hauggaard-Nielsen and Jensen 2001; Jensen 1996) and whereas an increase in the availability of N generally gives rise to increased biomass production, the degree of complementarity between component crops is often diminished as the legume
5 becomes increasingly suppressed by the cereal component (Midmore 1993; Ofori and Stern 1987). These studies clearly point at the significant role of the environment in modifying the competitive abilities of component crops. In agricultural research the study of non-legume holding mixtures has been very limited, however combining annual species with differences in length of their growing season has in terms of resource use been
10 succesful in a number of studies (Rerkasem *et al.* 1980; Trenbath 1974).

The aims of this study were to determine: i) how the productivity of dual- and tri-component annual intercrops, compared to that of the individual sole crops is influenced by the availability of N; ii) to determine the partitioning of / competition for soil and fertilizer
15 N among intercrop components including the recovery of fertilizer N; iii) to determine the effect of intercropping on N₂ fixation and ultimately to evaluate whether the complementarity of resource use (N) increases with the number of intercrop components.

Materials and methods

Site and soil

5 The field experiment was carried out from April to August 2000 at the experimental farm of the Royal Veterinary and Agricultural University, Denmark located 20 km west of Copenhagen (55°40'N, 12°18'E). The soil was a sandy loam with 18% clay, 18% silt, 55% finesand and 36% coarse sand, a pH (H₂O) of 6.7 and a 1,3% total C and 0.1% total N content in the topsoil (0-25 cm). The soil, sampled thirteen days after sowing, contained
10 0.34, 0.33 and 0.39 g KCl-extractable inorganic N m⁻² in the 0-20, 20-40 and 40-70 cm depths of the soil profile, respectively. In the two years preceding the trial red clover (1998) and spring barley undersown with rye grass (1999) were grown on the site. The soil contained efficient populations of *Rhizobium leguminosarum* bv. *viciae*. Data on daily rainfall, accumulated rainfall, 30-year average rainfall and average daily temperatures are
15 shown in figure 1.

Crop species and experimental design

Using a proportional replacement design *Pisum sativum* L. (field pea), *Hordenum vulgare*
20 L. (spring barley) and *Brassica napus* L. (oilseed rape) were grown as sole crops (SC), in dual-component intercrops and in a tri-component intercrop (IC), giving a total of seven crop treatments. Two levels of N fertilisation were employed, 0.5 and 4.0 g N m⁻² (N0 and N1, respectively). The experiment was organized as a randomized split-plot design with

fertilisation level as main plot factor, crop treatment as subplot factor with four replicates. Each subplot (18m²) consisted of ten rows of length 12m, spaced 12.5 cm apart.

Characteristics of component crops

5

The three crops were chosen on the basis of knowledge of their morphological and physiological differences, assuming that these would give rise to some degree of resource complementarity. The following cultivars were chosen: spring barley cv. Punto, a short cultivar that was included as an intermediately competitive barley cultivar, field pea cv.

10 Bohatyr, a tall, white flowered, full leafed cultivar with indeterminate growth as a competitive field pea cultivar and oilseed rape cv. Orakel, a hybrid and an early cultivar also considered to be competitive.

Crop management practices

15

The crops were sown on the 27th of April. Sole crop densities of 80 pea, 350 barley and 110 rape plants m⁻² were aimed at. The two and three component crop mixtures consisted of half and a third of the sole crop densities of each species, respectively. Pea, barley and oilseed rape seeds were sown consecutively in the same row, first the pea seeds were sown
20 at a depth of 6 cm, followingly barley seeds at 4 cm and lastly the rape seeds at a depth of 2 cm. Plant population densities and intercrop composition are given in table 1.

A ¹⁵N microplot holding 10 rows of 2.7 m length was placed within each subplot. These microplots received the same amount of urea-N as the subplots but in a ¹⁵N labelled form.

In the microplots the enrichment of the labelled urea was 2.5% and 5% for the N0 and N1 treatments, respectively. The ^{15}N enriched urea was dissolved in water and sprayed on silica sand while stirring the sand in a mixer. The treated sand was hand-spread as evenly as possible on the microplots, and immediately thereafter watered down with 2 L of tap
5 water (Høgh-Jensen and Schjøerring 1994). Plots were fertilised on the 10th of May.

Plant sampling and analytical methods

To determine the degree to which attempted intercrop proportions were achieved the total
10 number of emerged plants was determined in all plots, two weeks after emergence. A total of five sequential harvests were taken: 33, 42, 61, 72 and 112 days after sowing, respectively. At the first four harvests plant material was hand harvested from 0.5 m² of each subplot and from 1 m² at the final harvest. From the microplots two rows of 0.5 m
15 length were sampled at all five harvests. Harvested plant material was separated into component crops and individual biomass yields determined before and after drying at 80°C for 24 h. At the last harvest pods of pea and oilseed rape were divided into podwalls, grain and seed respectively and heads of barley divided into grain and glume before weighing.

Determination of nitrogen fixation and fertilizer recovery

20

The amount of atmospheric N₂ fixed was calculated as the product of pea biomass, % N content and the proportion of plant N derived from N₂ fixation (Ndfa). Similarly N derived from added fertilizer and the soil N pool was calculated as the product of plant biomass, %

N content and the proportion of N derived from added fertilizer (Ndff) and soil N (Ndfs), respectively.

Ndfa, Ndff and Ndfs were determined using well-known isotope dilution equations (Fried
5 and Middelboe 1977):

$$Ndfa(\%) = 100 \times \frac{\left((atom\%^{15}N_{excess_{Barley}} + atom\%^{15}N_{excess_{Rape}}) / 2 \right) - atom\%^{15}N_{excess_{pea}}}{\left((atom\%^{15}N_{excess_{Barley}} + atom\%^{15}N_{excess_{Rape}}) / 2 \right)} \quad (1)$$

$$Ndff(\%) = 100 \times \frac{atom\%^{15}N_{excess_{sample}}}{atom\%^{15}N_{excess_{fertilizer}}} \quad (2)$$

$$Ndfs(\%) = 100 - (Ndfa(\%) + Ndff(\%)) \quad (3)$$

where $atom\%^{15}N$ denotes the nitrogen isotope composition i.e. the ^{15}N /total N ratio and the $atom\%^{15}N$ excess is calculated as:

$$atom\%^{15}N_{excess} = (atom\%^{15}N_{sample}) - (atom\%^{15}N_{atmN_2}) \quad (4)$$

and the $atom\%^{15}N$ of atmospheric N_2 ($atm\ N_2$) = 0.3663

For the calculation of Ndfa, the average $atom\%^{15}N$ of reference plants (barley and rape) were assumed to provide a measure of the $atom\%^{15}N$ of soil N available to the legume
10 (Peoples *et al.* 1997). Before calculation the ^{15}N enrichments were corrected for seed N

assuming that 50% of the barley (1mgN/seed) and pea (10mgN/seed) seed N was present in harvested plant parts (Jensen *et al.* 1985).

Statistical analysis

5

Effects of crop treatment and fertiliser application were analysed using the GLM procedure available from SAS (Statistical Analysis System) with the following split-plot model (Searle 1971).

$$10 \quad X_{ijk} = \mu + \alpha_i + \gamma_j + (\alpha\gamma)_{ij} + \kappa_k + \eta_{ik} + \varepsilon_{ijk}$$

Where i, j and k refer to the whole-plot factor (N), split-plot factor (crop treatment) and block, respectively. $\kappa \sim N(0, \sigma^2_{\kappa})$, $\eta \sim N(0, \sigma^2_{\eta})$ and $\varepsilon \sim N(0, \sigma^2_{\varepsilon})$. The significance of difference between treatments were estimated using F-tests, probabilities equal to or less than 0.05 were considered significant. If analysis of variances showed significant treatment effects a least significant difference (LSD) test was used to compare treatment means.

15

Results

Aboveground biomass accumulation and grain yield

5 At both levels of nitrogen fertilisation, all sole- and intercrops display similar growth curves, producing comparable amounts of biomass in the beginning of the growth season, the greatest growth increments taking place in the period from 42 to 72 days after sowing and growth levelling off or even declining thereafter (figure 2). At both levels of N addition the pea sole crop produced the greatest amount of biomass in the interval from 42
10 to 72 days after sowing but due to a drop in total measured biomass in the last growth interval, the final yields of the pea sole crops only slightly exceeded those of the highest yielding intercrops. At the final harvest the biomass yields of the two nonlegume sole crops were significantly lower than those of the other crop treatments in the low N treatment and comparable to that of the pea-barley and barley-rape intercrops at the high N
15 level (table 2). Whereas the pea SC yield did not respond to N addition both non-fixing crops increased their yields significantly.

The greatest grain yields are reached in the sole crop pea treatments, the lowest in sole cropped rape and all intercrops and barley sole crops yielded intermediately (table 2). The
20 allocation of biomass to the grain fraction was, similarly to the total biomass production, unaffected by the level of N addition. The allocation of biomass to the grain fraction was proportional to the total biomass accumulated by a given crop (table 2).

Intercrop performance

Employing the LER (Land Equivalent Ratio) index (Willey and Osiru 1972) as a means of evaluating intercrop performance it was apparent that the benefit of intercropping over sole cropping was greater at the low than at the high level of N addition (table 2). Calculated on the basis of final aboveground biomass all intercrops, with the exception of the pea-barley combination displayed LER value of around 1.3 in the N0 treatments.

Abundance of component crops

On the basis of plant counts performed two weeks after germination it is clear that the intended relative proportion of component crops in the four studied intercrops was almost achieved (table 1). However, a slight dominance of barley in the barley-rape mixture was seen. With the exception of the barley-rape the relative biomass production of component crops changed greatly, from the first to the last harvest, in all intercrops at the high N level whereas the percentual distribution remained more or less constant at the low N level in all but the tri-component IC (table 3). The level of nitrogen fertilisation had a clear effect on the proportion of pea in all its mixtures at the final harvest, pea attaining a greater proportion at the low N fertilisation level. When the values for the percentage composition of component crops at the final harvest (table 3) were recalculated so that it was possible to compare the performance of the crops in the two- and three-component mixtures it became apparent that, relative to seed input, both pea and rape achieved the greatest yields in the pea-rape intercrop whereas for barley this was the case in the three-component IC (data not shown).

The pea component in all mixtures made up for a greater proportion of the grain yield than the total biomass yield. Barley made up for more than a proportionate part of the final biomass and grain harvested in all but the pea-barley treatment.

5

N accumulation and grain N-yield

At both levels of N fertilisation nitrogen was taken up at a steady rate from the beginning of the growth period till the last studied growth interval (day 72 to 112 after sowing) where
10 the net uptake appeared to level off in all but the pea-barley-rape and barley-rape intercrops at N0.

As for all other yield parameters measured, grain N content was greatest for sole cropped pea, irrespective of N fertilisation level. The lowest concentrations of grain N were
15 measured in the non-legume holding sole- and intercrops, other intercrops yielded intermediately (table 2). Similarly to the total accumulation of N, allocation of N to the grain fraction was unaffected by the level of N addition.

Uptake of soil- and fertiliser-N
20

In all mixtures barley was the most efficient competitor for soil N, accounting for the main part of the accumulation (figure 4). However with respect to fertiliser N the situation was somewhat different, the rape component accumulated comparable or only slightly lower amounts of fertiliser N than barley in the barley-rape and triple intercrop. When
25 intercropped, both non-legumes are more efficient soil and fertiliser N scavengers than pea,

however when sole cropped the pea crop took up comparable amount of both soil and fertiliser N as sole cropped barley and rape. As would have been expected a greater fertiliser N uptake was observed for all crop treatments when N fertilisation was increased from 0.5 to 4.0 g N m⁻². However increased fertilisation did not give rise to a significant
5 increase in the total uptake of soil N (figure 4).

At both levels of fertilisation the lowest recovery of added fertilizer N was measured for the pea-barley intercrop. For all other crop treatments the recovery exceeded or equaled 50% at NO and exceeded 30% at N1 (table 5).

10 Symbiotic N₂ fixation

At both levels of N addition the largest amount of N₂ was fixed by pea when sole cropped (figure 5). At the low fertilisation level the impact of competition from non-legumes had a promoting effect on the N₂ fixation process of pea, %Ndfa exceeding that determined for
15 the pea sole crop at all harvests. At the high fertilisation level competition from the non-legumes had less of an effect, the differences between the sole crop and intercrop fixation percentages rarely being significantly different. However at the final harvest an effect was clearly prevalent.

Discussion

Diversity and crop performance - total biomass and grain yield

5 Annual intercrops have been reported to be more productive than comparable sole crops (Hauggaard-Nielsen *et al.* 2001a; Hauggaard-Nielsen and Jensen 2001; Jensen 1996; Ofori and Stern 1987; Willey and Osiru 1972). Results from the present study indicate that this may not always be the case since sole cropped pea was the highest yielding crop (figure 2). Pea is known to be a variable crop, a variability that among many factors is linked to its
10 drought sensibility, especially during flowering and in the early pod-filling growth stage (Jensen 1997, Monti *et al.* 1994). Seen as a whole the growth season of 2000 experienced average rainfall conditions (figure 1), however large amounts of precipitation prior to flowering may have ensured a good supply of soil water and therefore be the primary reason why sole crop pea yields were high.

15

The three crops that were included in the study were chosen on the basis of assumed differences in their response to the growing environment. Among these the ability of pea to fix atmospheric nitrogen; the ability of barley and pea, unlike rape, to enter into a mycorrhizal symbiosis and differences in the phenological development of the three crops,
20 the time from germination to maturity of rape exceeding that of barley and pea.

Differences that were considered important for achieving complementary use of growth resources. As is apparent from calculated LER values complementarity was apparent in all intercrops at the low fertilisation level and even with increased N fertilisation the pea-rape and pea-barley-rape intercrops had LER values exceeding unity (table 2). It is commonly

recognized that the ability of legume and nonlegume to exploit different N pools frequently leads to yield advantages over their component sole crops, and more so than combinations of nonlegumes (Vandermeer 1989). It was surprising that the LER of the barley-rape mixture was comparable to that of the legume holding intercrops at the low level of N addition, indicating the presence of complementarity between the two non-legumes, complementarity that appeared to be lost when the fertilisation level was increased. As discussed by Fukai and Trenbath (1993) the application of a limiting resource, in this case nitrogen, would be expected to favour the growth of the dominant crop component, thereby negatively affecting the growth of the suppressed component. This could clearly explain the observation that barley gained on account of rape when the availability of fertiliser N increased.

The benefits of intercropping, evaluated as the size of calculated LER values, were clearly diminished by increased N addition, similar observations were made by (Hauggaard-Nielsen *et al.* 2001a; Ofori and Stern 1987; Waterer *et al.* 1994). Ofori and Stern (1987) reviewed the influence of applied N on various intercropping systems. They found that intercrop cereal yields increased progressively with N application, while yields of the legume either decreased or responded less. In the present study the legume fraction of the intercrops was negatively affected by the addition of N whereas both rape and barley responded positively indicating that the performance of pea is decisive for the intercrop advantages obtained. Rauber *et al.* (2001) compared the suitability of several pea cultivars as components of legume-cereal intercrops and found the performance of pea to be positively related to the grain yield production of the mixtures and the magnitude of calculated relative yield total (RYT), a measure similar to the above mentioned LER.

On the basis of the amount of biomass allocated to grain in the three sole crops (64, 60 and 35% in pea, barley and rape, respectively), the observed partitioning of total biomass between vegetative and reproductive structures in the four intercrops was not surprising, reaching intermediate values (60, 50, 52 and 54% in pea-barley, pea-rape, barley-rape and pea-barley-rape, respectively). This indicates that for all three crops the allocation of biomass to the grain is unaffected by the nature of the companion crop(s) and the availability of fertiliser N.

10 Diversity and crop performance - total N and grain-N yield

Total N accumulated by the crop treatments paralleled total biomass yields, all pea containing crop treatments accumulating more N than both the barley and rape sole crops and their combined intercrop (figure 3). The three sole crops displayed clear differences in their allocation of accumulated N to the grain fraction, differences that, when the relative proportion of component crops was taken into account explained differences in allocation between intercrops.

Although not significant, increased N addition gave rise to decreases in the total N yields of pea-barley and pea-rape dual intercrops (table 2). Decreases that were paralleled by declines in the proportion of pea in the final pool of biomass harvested relative to the biomass harvested 33 days after sowing (table 3), the proportion of pea falling from 45 to 31 and 61 to 43 in association with barley and rape, respectively. This indicates that elevating the N fertilisation level gave rise to a competitive suppression of pea, which in

turn had a direct effect on the amount of N being accumulated, most likely due to reduced N₂ fixation. In the tri-component intercrop the pea component was equally suppressed by the joint action of the two non-legumes however N yields were maintained or even slightly improved implying that the two nonlegumes complemented one another with respect to N uptake.

Apparently intercropped rape, irrespective of intercrop treatment, continued to accumulate N between the last two harvests, whereas uptake levelled off for both pea and barley (data not shown). Since the rape sole crops displayed similar accumulation patterns it is likely that the developmental time and pattern of N uptake of rape was different from that of both the pea and barley. Whatever the explanation these differences demonstrate the potential of rape holding intercrops to bring about more efficient resource use over time.

Competition for and accumulation of soil N

Despite accounting for approximately half of the total biomass production, pea accumulated much less soil N when intercropped than could have been expected from sole crop uptake (figure 5A). This clearly emphasizes the competitive superiority of rape and barley when focus is on soil N. Barley was the most competitive, accounting for more than a proportionate part of the total N accumulated in all intercrops of which it was a part. Jensen (1996) observed a similar superiority of the barley component of intercrops of pea and barley. This dominance was not predictable on the basis of the performance of the three crops in sole crop, where both pea and rape accumulated more soil N than barley, however in the initial growth phase, the common observation is that one species grows faster than the other(s), progressively leading

to dominance in terms of resource acquisition and thus to greater biomass growth and yield (Fukai and Trenbath 1993). In a model-based study, early emergence and rapid growth in the first stages of development were found to increase competitive advantage of species (Radosevich and Roush 1990). The barley crop grew faster initially than rape, an early
5 advantage that resulted in the accumulation of a more than proportionate part of the soil N acquired by the intercrops holding these two components (table 2). In association with pea barley did not dominate to the same degree but nevertheless accounted for a significantly greater proportion of soil N uptake. The data indicates that an initial competitive advantage of barley in the two dual intercrops was strengthened by the addition of N, the barley component
10 accounting for a greater relative proportion of soil N accumulated at the high N level. In the triple intercrop dominance of barley experienced by pea and rape was less strong and was not strengthened by the addition of N, indicating that the presence of multi-species interactions diminished the impact of the dominant. The presence of more than two crops in an intercrop opens for the possibility of indirect facilitation. As discussed by (Vandermeer 1989) one
15 component, call it A may have a positive indirect effect on component B through its competitive effect on component C. The depression of barley in the tri-component mixture may well be the result of the bettered growth of both rape and pea as a result of indirect facilitation.

20 With respect to soil N uptake and fertilizer N uptake pea was clearly severely suppressed by both nonlegumes, however more so by barley than rape. A greater accumulation of both soil and fertiliser N in the pea-rape intercrop compared to the pea-barley intercrop could in accordance with (Fukai and Trenbath 1993) indicate that the increased performance of a weak competitor may increase intercrop performance.

Competition for and accumulation of fertiliser N

Increasing the fertilisation level from 0.5 to 4.0 g N m⁻² naturally led to an increase in the
5 uptake of fertiliser derived N by all studied crops (figure 5B). The relative increase in
fertiliser uptake was similar for barley grown as a sole crop and in dual intercrop with
either pea or rape. However the uptake of both rape and pea responded differently when
intercropped with barley than in the other crop treatments. For both rape and pea the
10 relative uptake of fertiliser N increased only 3 fold when grown in dual intercrop with
barley whereas in joint association and as sole crops the uptake increased more than 5 fold.
This clearly emphasizes that the competitive impact of barley on rape and pea.

Whereas barley was a stronger competitor than rape, rape was clearly less suppressed when
competition was for fertiliser N than soil N. Part of the explanation for this may be that
15 faster initial root growth gave barley an advantage in the pursuit of soil N, gaining access
to pools in deeper soil layers than rape. This morphological advantage may be assumed to
have been of lesser importance when competition was for fertiliser N as this was primarily
available in the upper layer of the soil profile.

20 Recovery of added fertiliser N was significantly lower at the high level of fertilisation (table
5), which could indicate that the addition of 5 kg ha⁻¹ to a greater extent matched the
demand of the crops than was the case at the higher fertilisation level. Whereby losses via
ammonia volatilisation, as a result of the hydrolysis of the added urea, may to a greater
extent have been minimised.

Effect of intercropping on fixation

In line with observations made by (Starling *et al.* 1998; Waterer and Vessey 1993) the
5 addition of 40 kg N ha⁻¹ enhanced the fixing capacity of the sole cropped pea, an
enhancement that persisted until the final harvest (table 6). Implying that a greater
availability of easily accessible N improved the conditions of establishing the N₂ fixing
apparatus. For intercropped pea the percentage of fixation in the low fertilisation
10 treatments exceeded those measured at the high N level throughout the study, indicating
that the promoting effect of competition from companion crops on the fixation process was
strongest under low N fertilisation conditions.

As noted earlier the competitive pressure exerted by barley towards pea was clearly
stronger than that of rape on pea when focus was on fertiliser and soil N. At the same time
15 the greatest relative amount of N₂ fixed at maturity was measured for the pea grown in
association with rape under conditions of low N fertilisation whereas the two other pea-
holding intercrops fixed amounts comparable to the pea sole crop (figure 4). This may
indicate that the benefits achieved from the association of a legume and nonlegume are
partly lost if the nonlegume is too strong a competitor for soil N. As previously observed
20 by (Ofori and Stern 1987) the potential of the intercropping practice, as a means of
increasing the contribution of N derived from atmospheric fixation was lost as fertilisation
level was increased, the relative amounts of N₂ fixed by pea in all intercrops being lower
than could have been expected from the sole crop.

Intercrop species richness, productivity and N use

The diversity of opinion about the functions of diversity in agricultural cropping systems is high while the data on which a solid judgement could be formulated remains sparse (Giller *et al.* 1997; Swift and Anderson 1993; Vandermeer *et al.* 1998). However many seem to agree that crop-species composition and diversity may among other things profoundly affect soil fertility (Hooper 1998; Russell 2002; Swift and Anderson 1993), increase nutrient and water-use efficiency and resistance to crop diseases (Mitchell *et al.* 2002), thereby providing stability to the cropping system (Swift and Anderson 1993; Trenbath 1999). Results from natural ecosystem studies further indicate that increased diversity of species may give rise to increased productivity (Tilman *et al.* 2001; Tilman *et al.* 1996). The reasons for this link are still being debated, be it the result of increased probability of including keystone species or functions as diversity increases (Hooper 1998) or a more intricate effect of diversity per se (Tilman *et al.* 2001). As has been the focus of the present study most species-diversity studies have focussed on short term effects of low diversity (2-3 species) systems, often dominated by N₂ fixers. Clearly these are low diversity systems and one may, with right, ask whether these studies shed light on the link between diversity and ecosystem functioning? However if species composition and the presence of specific functions are as important as species richness then studying the effects of increasing the number of species in a crop from 1 to 2 to 3 may provide valuable insight. In this study we anticipated that because of differences in structural and biogeochemical traits, the three studied crops would use limiting soil resources in a complementary way giving rise to a greater biomass productivity when diversity of the cropping treatment was increased. However, we did not find an absolute increase in productivity when species

number was increased from 1 to 2 to 3 components. Parralleling this, results of numerous competition experiments, among these many intercropping studies (Trenbath 1974; Vandermeer 1990), lead to the conclusion that plant diversity does not necessarily result in absolute increases in net primary production, absolute yields frequently falling between those of the least and most productive species grown as sole crops (Hooper 1998). Nevertheless all intercrops, irrespective of composition displayed LER values close to or exceeding unity, indicating the complementary use of resources. The complementarity of the pea containing intercrops was to a certain degree the result of N-use complementarity through the ability of pea to fix atmospheric nitrogen. Overall N uptake of the studied cropping treatments was clearly influenced by the presence of pea, all pea containing crop treatments accumulating more N than barley and rape sole crops as well as their combined intercrop. Furthermore the pea crop proved valuable for obtaining a high quality of the harvested yield, the N content of the total grain fraction standing in direct relation to the proportion of pea in a given crop treatment. In the barley-rape intercrop LER values comparable to those of the pea mixtures indicate that these two non-legumes clearly complemented one another in some way or another. Complementarity was not for total N-uptake since uptake by the intercrop did not differ significantly from that of the two sole crops however differences in the temporal development of the two crop species, leading them to complement each other over time may explain the greater relative yield of the intercrop.

This study clearly points at some of the potential advantages of increasing the diversity of intercrops, be they through complementary use of N (through a N₂ fixer), differences in phenomolgical development. Whether there are clear advantages of increasing the number

of component crops from 2 to 3 species is not clear, but it is apparent that the competitive and complementary interactions between species are altered by the presence of an additional crop component. The results of the present study emphasize the importance of initial population dynamics for structuring intercrop composition and the prevailing
5 patterns of dominance and suppression.

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Table 1. Plant populations in sole- and intercrops of pea, barley and rape two weeks after seedling emergence. Values are the mean (n=8).

Crop	Plant population (plants m ⁻²)			Intercrop composition ^b		
	Pea	Barley	Rape	% Pea	% Barley	% Rape
Pea SC	75 (±5)					
Barley SC		312 (±10)				
Rape SC			100 (±10)			
Pea-Barley IC	42 (±4)	172 (±5)		52	48	
Pea-Rape IC	33 (±4)		50 (±4)	51		49
Barley-Rape IC		167 (±6)	43 (±6)		58	42
Pea-Barley-Rape IC	28 (±3)	128 (±6)	38 (±7)	35	35	30

^b Calculations based on plant units, e.g. 1 unit = 1 pea plant, 4.5 barley plants or 1.6 rape plants.

Table 2. Total crop DM yield, grain yield, total crop N yield and grain N yield of pea, barley and rape in sole- and intercrops and Land Equivalent Ratio (LER) calculated on the basis of total crop DM yields. Values are the mean (n=4).

Crop	N fertilizer treatment	Yield		N yield		LER
		Total crop g DM m ⁻²	Grain	Total crop g N m ⁻²	Grain	
Pea SC	N0	615	394	18.9	16.5	
	N1	610	388	18.7	16.2	
Barley SC	N0	352	210	4.1	3.1	
	N1	482	287	5.5	4.1	
Rape SC	N0	312	112	5.3	3.7	
	N1	448	157	7.5	5.3	
Pea-Barley IC	N0	496	307	10.8	9.1	1.10
	N1	513	307	8.9	7.1	0.99
Pea-Rape IC	N0	571	294	14.3	11.7	1.32
	N1	588	278	13.3	10.4	1.16
Barley-Rape IC	N0	454	242	5.9	4.4	1.33
	N1	462	241	6.5	4.6	0.97
Pea-Barley-Rape IC	N0	507	286	9.6	7.7	1.26
	N1	575	306	10.5	8.1	1.15
LSD(0.05) _{crop}		71	45	2.1	1.8	

Table 3. Percentual distribution of component crops (pea, barley and rape) in total biomass harvested 33 days after sowing (harvest 1) and final harvested biomass. Values are the mean \pm s.e. (n=4).

Crop	N fertilizer treatment	% of harvest 1			% of final yield		
		Pea	Barley	Rape	Pea	Barley	Rape
Pea-Barley IC	N0	48 (\pm 3)	52 (\pm 3)		52 (\pm 3)	48 (\pm 3)	
	N1	45 (\pm 2)	55 (\pm 2)		31 (\pm 5)	69 (\pm 5)	
Pea-Rape IC	N0	57 (\pm 6)		43 (\pm 6)	56 (\pm 3)		44 (\pm 3)
	N1	61 (\pm 6)		39 (\pm 6)	43 (\pm 5)		57 (\pm 5)
Barley-Rape IC	N0		77 (\pm 4)	23 (\pm 4)		77 (\pm 7)	23 (\pm 7)
	N1		76 (\pm 2)	24 (\pm 2)		78 (\pm 6)	22 (\pm 6)
Pea-Barley-Rape IC	N0	27 (\pm 3)	50 (\pm 3)	23 (\pm 3)	34 (\pm 1)	50 (\pm 1)	16 (\pm 1)
	N1	38 (\pm 3)	50 (\pm 1)	12 (\pm 4)	23 (\pm 6)	55 (\pm 8)	22 (\pm 2)

Table 4. Percentual distribution of component crops (pea, barley and rape) in the final grain yields, calculated on a weight basis. Values are the mean \pm s.e. (n=4).

Crop	N fertilizer treatment	% of final grain yield		
		Pea	Barley	Rape
Pea-Barley IC	N0	55 (\pm 3)	45 (\pm 3)	
	N1	33 (\pm 6)	67 (\pm 6)	
Pea-Rape IC	N0	70 (\pm 2)		30 (\pm 2)
	N1	55 (\pm 6)		45 (\pm 6)
Barley-Rape IC	N0		85 (\pm 5)	15 (\pm 5)
	N1		86 (\pm 5)	14 (\pm 5)
Pea-Barley-Rape IC	N0	40 (\pm 2)	51 (\pm 1)	9 (\pm 1)
	N1	27 (\pm 7)	60 (\pm 10)	13 (\pm 3)

Table 5. Recovery of added fertilizer N for all 7 crop treatments fertilised with 0,5 g N m⁻² (N0) and 4 g N m⁻² (N1). Values are the mean ± s.e. (n=4).

Crop	Recovery (%)	
	N0	N1
Pea SC	58 (±13)	38 (±5)
Barley SC	50 (±3)	32 (±3)
Rape SC	50 (±11)	37 (±5)
Pea-Barley IC	48 (±6)	26 (±3)
Pea-Rape IC	65 (±16)	44 (±1)
Barley-Rape IC	69 (±16)	37 (±5)
Pea-Barley-Rape IC	63 (±5)	37 (±9)

Table 6. Percent of nitrogen uptake derived from atmospheric nitrogen fixation (%Ndfa) for solecropped (SC) pea and the pea component of intercrops (IC).

Crop treatment	N fertilizer treatment	Days after sowing				
		33	42	61	72	112
Pea SC	N0	58	63	59	79	76
	N1	61	78	63	88	69
Pea-Barley IC	N0	81	87	82	86	86
	N1	77	76	76	81	85
Pea-Rape IC	N0	69	78	85	86	87
	N1	67	70	77	77	73
Pea-Barley-Rape IC	N0	85	81	91	91	84
	N1	66	76	70	80	87
LSD (0.05) _{crop}		11.1	8.0	13.8	7.6	5.3

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Figure legends

Figure 1. Year 2000 and the 25 year average daily temperature and 30 year average rainfall. Measured at the experimental farm of the Royal Veterinary and Agricultural University, Denmark. Time of plant developmental stages indicated with arrows.

Figure 2. Total dry matter production (g DM m^{-2}) in sole- and intercrops of pea, barley and rape, at two levels of N addition 0.5 g N m^{-2} (N0 - open symbols) and 4.0 g N m^{-2} (N1 – closed symbols). Values are the mean ($n=4$). Corresponding to each harvest $\text{LSD}_{(0.05)}$ between crop treatments are at each N level given by bars.

Figure 3. Land Equivalent Ratio (LER) calculated on the basis of total crop DM yields for all intercrops of pea, barley and rape, at two levels of N addition 0.5 g N m^{-2} (N0 – black bars) and 4.0 g N m^{-2} (N1 – grey bars).

Figure 4. Total N accumulation (g N m^{-2}) in sole- and intercrops of pea, barley and rape, at the two levels of N addition 0.5 g N m^{-2} (N0 - open symbols) and 4.0 g N m^{-2} (N1 – closed symbols). Values are the mean ($n=4$). Corresponding to each harvest $\text{LSD}_{(0.05)}$ between crop treatments are at each N level given by bars.

Figure 5. Soil and fertilizer N uptake (g N m^{-2}) by sole- and intercrops of pea, barley and rape, at two levels of N addition 0.5 g N m^{-2} (N0 - open symbols) and 4.0 g N m^{-2} (N1 – closed symbols). Each column is split into the number of

components of the crop treatment. For all intercrops expected total crop treatment uptake, calculated on the basis of solecrop yields, is shown. Values are the mean (n=4). SE (bars) are given for total crop treatment uptake. $LSD_{(0.05)}$ for total crop uptake is indicated by bars.

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Figure 6. Total N accumulated from N_2 fixation ($g\ N\ m^{-2}$) by pea solecropped, in dual and tri-component intercrops with barley and rape, at two levels of N addition $0.5\ g\ N\ m^{-2}$ (N0 - open symbols) and $4.0\ g\ N\ m^{-2}$ (N1 – closed symbols). Values are the mean (n=4). Corresponding to each harvest $LSD_{(0.05)}$ between crop

10 treatments are at each N level given by bars.

Figure 1

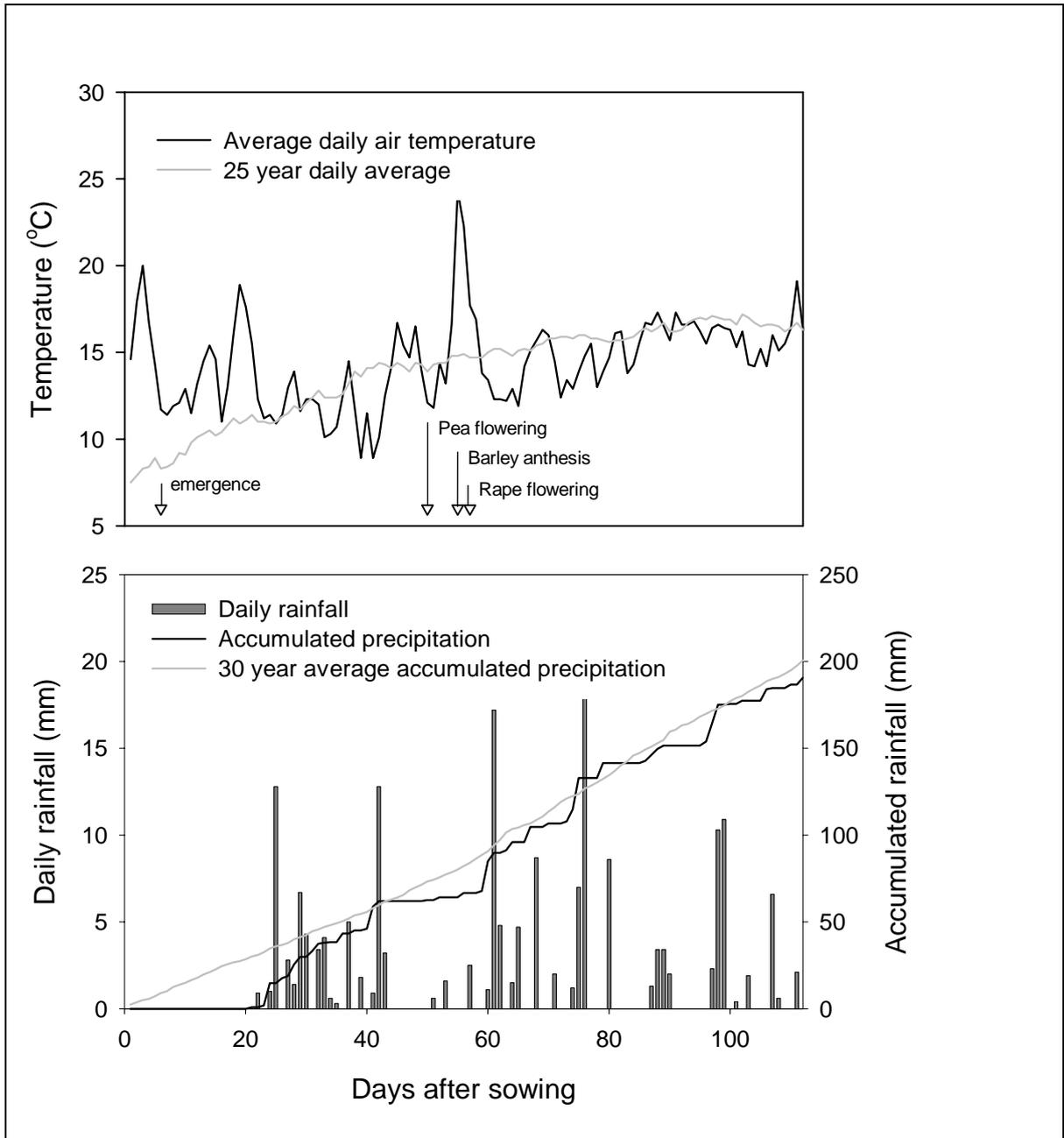


Figure 2

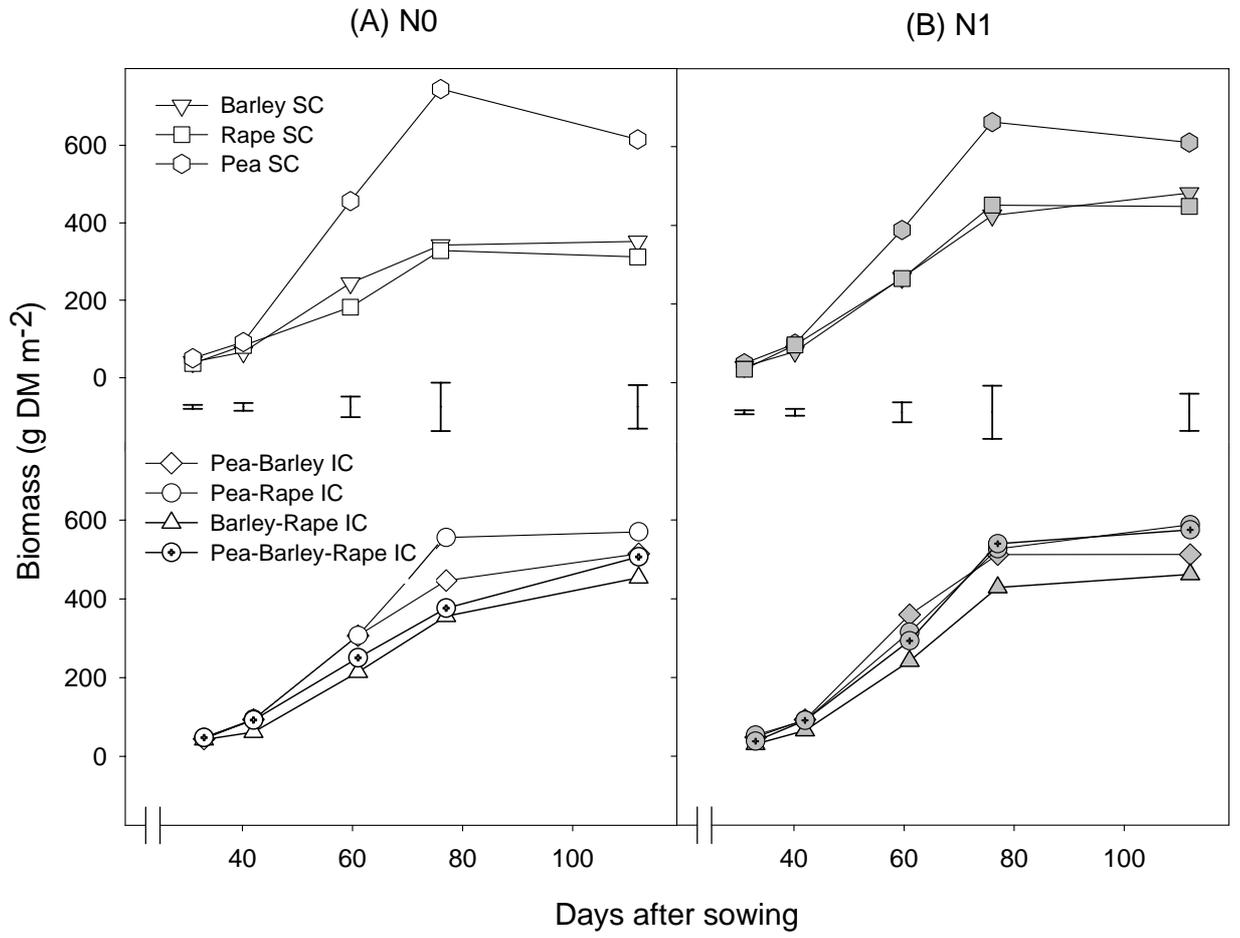


Figure 3

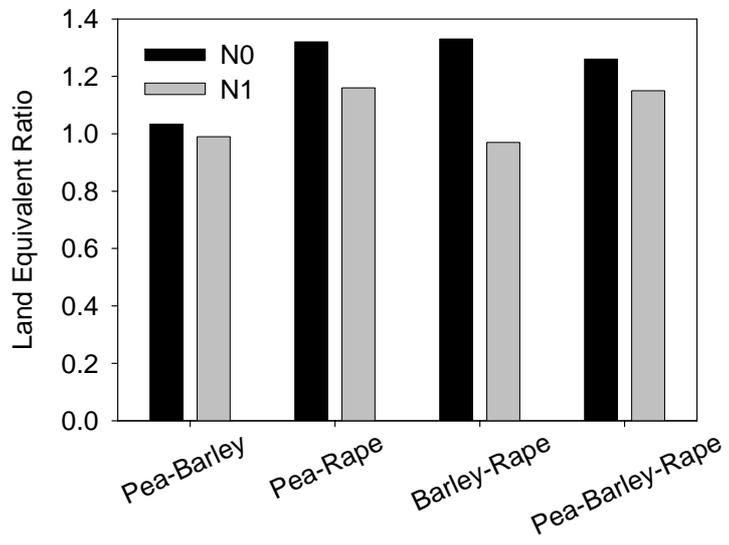


Figure 4

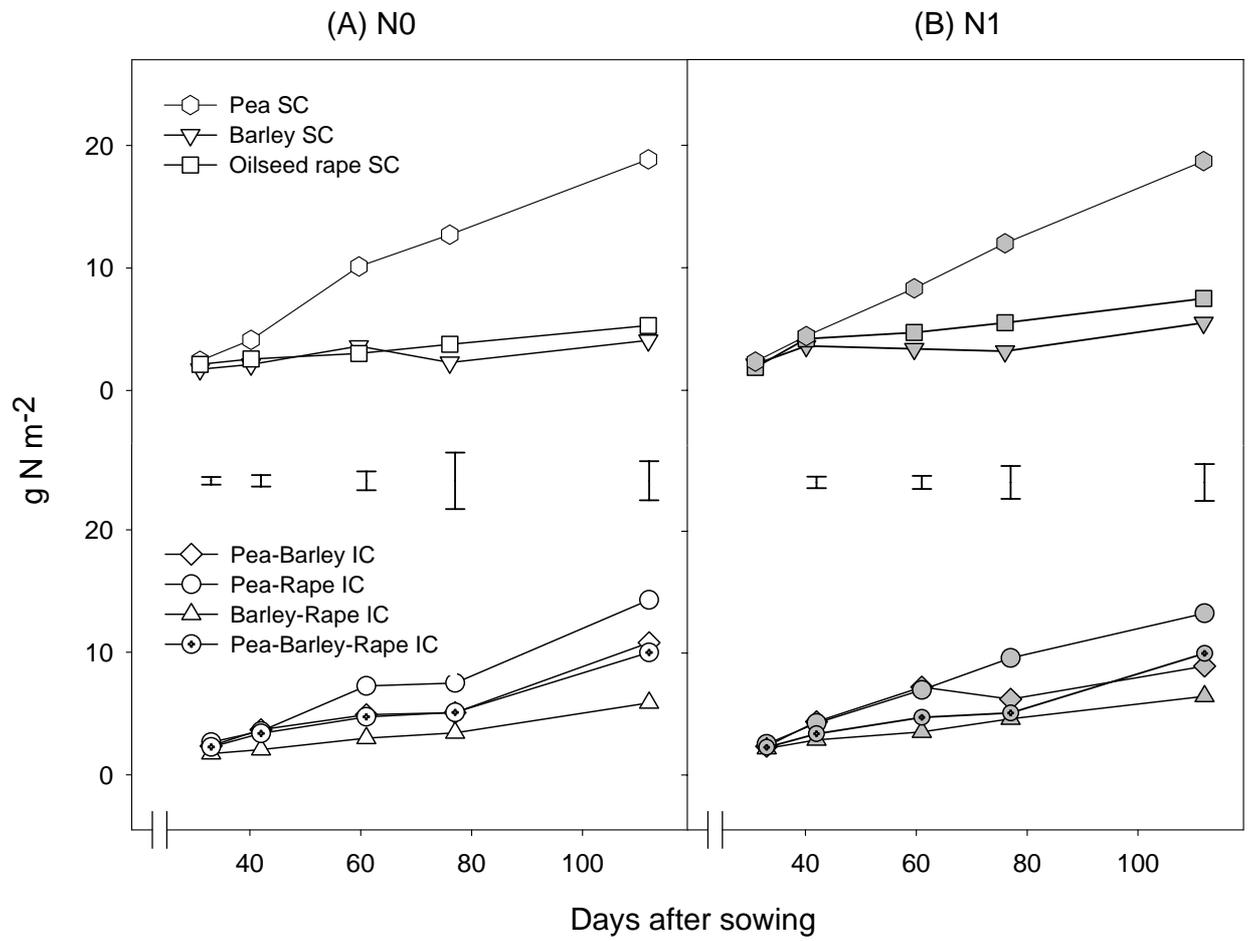
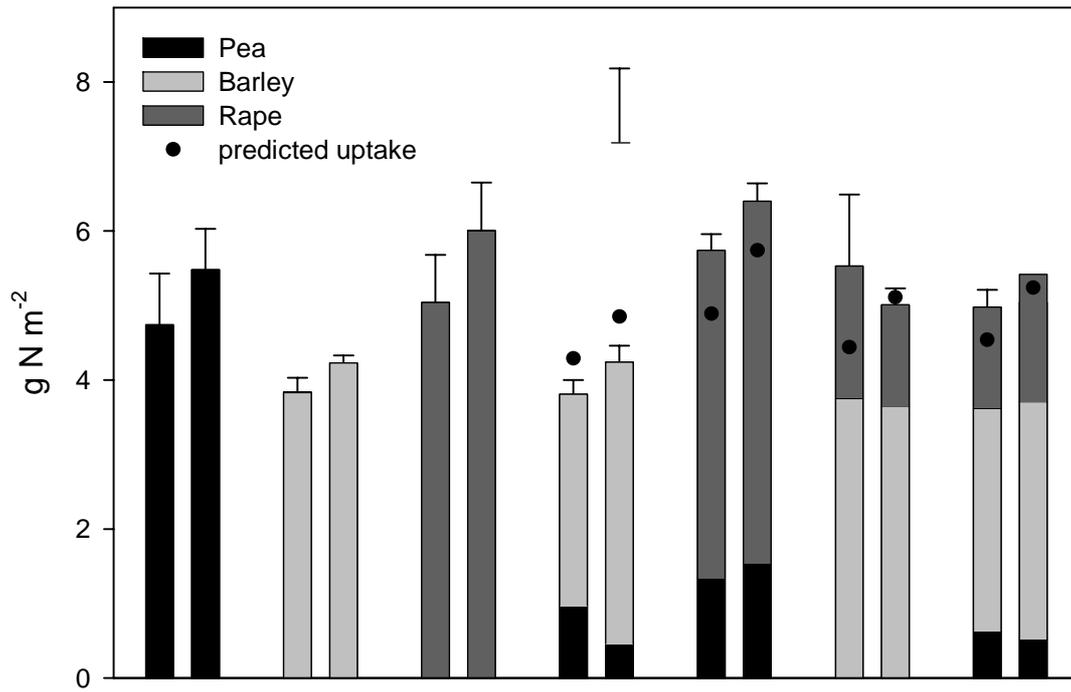


Figure 5

(A) Soil N



(B) Fertilizer N

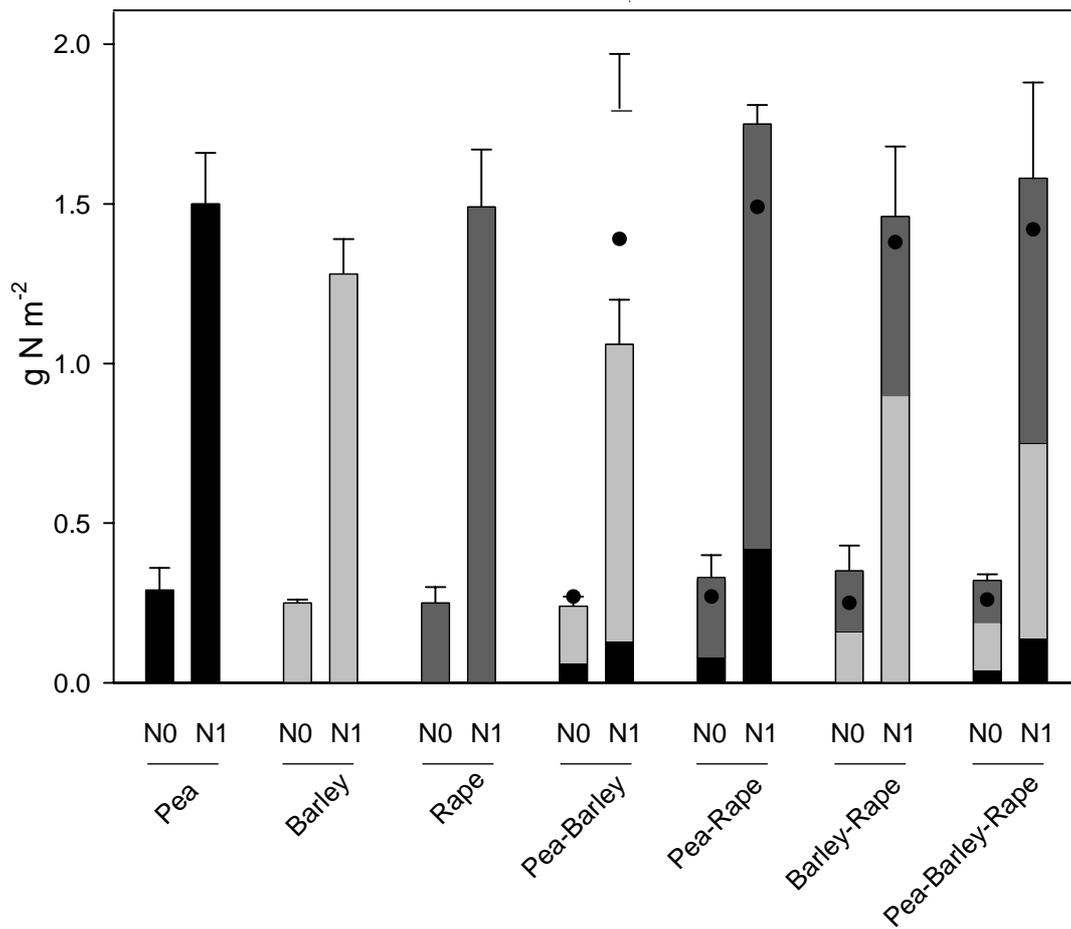


Figure 6

