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Effect of soil fertility level on growth of cover crop mixtures and residual fertilizing value for spring barley

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ABSTRACT

The use of cover crops may play an important role in ecological intensification of farming systems through their impacts on cycling and the availability of essential nutrients. However, little is known about the effect of soil fertility on the performance of cover crops and consequent nutrient dynamics. We performed a two-year field trial to quantify the effect of two soil fertility levels (low and moderate) on the growth and nutrient content of different leguminous and non-leguminous cover crop species and their mixtures, and on their fertilizing value for the succeeding crop.

Overall, the growth and shoot nutrient content of cover crops in autumn was influenced by the species, mixture choices and soil fertility level. The growth of some species was less affected by fertility level than others. Among the legumes, fertility levels had no effect on the growth of lupin. Among the non-legumes, buckwheat had the highest biomass production as well as C:N ratio at a low fertility level, and in the first year was affected least by soil fertility level. However, buckwheat and lupin grown as a single species or in mixtures did not lead to yield improvements in the subsequent crop. Growing a mixture of vetch and radish achieved the best synergetic effect and highest shoot N content. In both years, the greatest improvements in barley yields were achieved after vetch grown as a single species and in a mixture with radish (by up to 46 %). Rye was less competitive in mixtures than radish and buckwheat, and in the second year it had a negative effect on barley yield at the low soil fertility level, presumably due to pre-emptive competition for nitrogen. Soil fertility level had a greater effect on barley yields than cover crop treatments, as yield increments at the moderate fertility level amounted to 48 % in the first year and 64 % in the second year compared with the low fertility level.

These results demonstrate that the cultivation of winter-persistent legumes alone or in a mixture with oilseed radish offers a promising tool for improving the yields of subsequent crops through nitrogen input and nutrient cycling on farms where soil fertility level is low.

1. Introduction

Ecological intensification, i.e. improving crop yields without increasing external inputs by enhancing resource use efficiency and minimizing environmental impacts, is crucial for the sustainability of farming systems that rely more on internal nutrient cycling and less on external nutrient inputs. The use of cover crops, including leguminous as well as non-leguminous species, may play an important role in ecological intensification through their impacts on the availability of nitrogen (N) and other essential nutrients such as phosphorus (P) and sulfur (S) (Eriksen et al., 2004; Doltra and Olesen, 2013; Hallama et al., 2019; Hansen et al., 2021).

Cover crops may offer multiple additional benefits for biodiversity, pest and disease pressure, nutrient leaching, soil structure and carbon storage (Blanco-Canqui et al., 2015) and may therefore contribute to maintaining or enhancing both soil fertility and health (Abdalla et al.,...
Soil fertility affects plant growth and productivity directly through nutrient supply and indirectly through its influence on root growth and water supply. Interactions between soil fertility and cover crop growth may therefore occur (Blesh, 2018). Sufficient or high soil fertility supports optimal biomass production and nutrient accumulation in cover crops, which in turn have the potential to enhance soil fertility and functioning (Mortensen et al., 2021), while low soil fertility may limit cover crop growth and hence become a barrier to improving soil fertility. However, there is a high variability in nutrient status and soil fertility among farming systems, especially on organic farms, depending on the farm type and nutrient budget. Organic dairy farms generally have a nutrient surplus due to inputs from feed imports and a high proportion of forage legumes in the crop rotation, whereas intensive organic vegetable farms often have a surplus due to large application of manure or other organic fertilizers such as composts. Stockless organic arable farms with little or no external input often have nutrient deficits, especially in P and K (Cooper et al., 2018; Reimer et al., 2020b). Reimer et al. (2020a) report that arable organic farms with a high share of N from biological N fixation have a higher risk of deficits in other nutrients, especially P, K, Mg and S. If the nutrient status and soil fertility are low, most cover crops would be expected to grow poorly and be less able to enhance soil fertility. Thus, negative consequences for cover crop management on arable farms with limited access to external nutrient inputs may be expected in the longer term. Cover crop species that are able to grow well in soil with low fertility could alleviate the long-term decline and help improve soil fertility in low input systems by assimilating N, P, S and other nutrients, which in turn can be available for subsequent crops (Hansen et al., 2021).

Growing cover crop mixtures instead of single species may maximize the benefits of each species due to the principle of complementarity (Tribouilloy et al., 2016). Positive synergies of cover crop mixtures can result in higher biomass production compared with growing single species (over-yielding). Positive synergy through facilitation have been observed in mixtures of legumes and non-legumes (Tribouilloy et al., 2016; Wendling et al., 2017). For example, higher N uptake in mixtures than in single stand cover crop was observed in study of (Bessler et al., 2012) due to N fixation by legumes and high biomass productivity and N uptake by non-legumes. Negative effects of mixtures may occur due to competition between the cover crop species, resulting in decreased performance in mixtures compared with single species (Tribouilloy et al., 2016; Wendling et al., 2017; Couiedel et al., 2018). Combining complementary cover crop traits, such as root architecture, nutrient acquisition or residue quality, can increase the functional trait diversity and thereby multifunctionality in cover crop mixtures (Blesh, 2018). The interaction in mixtures is affected by both the species and soil fertility. Generally, grasses and cruciferous cover crops are more competitive when there is high soil N availability, whereas legumes may be more competitive at low soil N availability (Blesh, 2018; Langelier et al., 2021). However, knowledge about the effects of the soil availability of nutrients other than N on cover crop development and the subsequent residual nutrient effect is still scarce.

Cover crops can increase, reduce or have no impact on subsequent crop yields depending on the cover crop species, climatic conditions, soil type and management strategies (Doltra and Olesen, 2013; Blanco-Canqui et al., 2015; Valkama et al., 2015). Non-legume cover crops are primarily grown to retain soil mineral N and thereby reduce N leaching losses, whereas legumes that can fix atmospheric N typically have a higher N concentration and lower C/N ratios, and release more N than non-legumes after incorporation (Li et al., 2015b; Tribouilloy et al., 2016). Therefore, legumes have the potential to increase the yield of subsequent crops to a greater extent than non-legumes (Li et al., 2015a; Langelier et al., 2021). Besides N, cover crops can also potentially contribute to S and P nutrition of the succeeding crop by decreasing S leaching and increasing S and P availability, which again depends on the quality of cover crop residues and management (Eriksen et al., 2004; Damon et al., 2014). Growing mixtures of legumes and non-legumes as cover crops is therefore a promising strategy to optimize these ecosystem services (Tribouilloy et al., 2016). The integrative effect of biomass production, nutrient uptake and the fertilizing effect of cover crops and their mixtures depends on soil fertility. However, knowledge about the effect of soil fertility on cover crop performance is limited. Furthermore, improved understanding of the interspecific interactions of mixtures is needed in relation to different species and also soil fertility.

The overall aim of this two-year field study was to quantify the effect of soil fertility level on the growth, nutrient content and residual effect of different leguminous and non-leguminous cover crop species and their mixtures on the subsequent crop. The specific objectives were: (1) to evaluate the ability of specific cover crop species to acquire nutrients and produce biomass at a low soil fertility level, (2) to determine whether cover crop mixtures with legumes result in synergy/over-yielding regardless of soil fertility level, and (3) to identify the interaction between soil fertility level, cover crop performance and the grain yield of the succeeding barley crop.

2. Materials and methods

2.1. Experimental design

The field study was conducted at the Long-Term Nitrient Depletion Trial (LNTNDT) field established in 1964 at the University of Copenhagen’s research farm in Taastrup, Denmark (55° 40’ N, 12° 17’ E). The soil is a sandy loam and contains 16.4 % clay (<0.002 mm), 17.3 % silt (0.002–0.02 mm), 33.3 % fine sand (0.02–0.2 mm) and 31.2 % coarse sand (0.2–2 mm). Details of the experimental history, design and management practices have been described by van der Bom et al. (2018). Briefly, the LNTNDT design consists of 14 different fertilizer treatments, each represented in 4 replicate blocks (individual plot size 50 x 20 m). The cropping prehistory of the field trial before the current cover crop trial followed a three-year crop sequence consisting of spring wheat (Triticum aestivum) L. cv. KWS Bittern sown in spring 2016, winter wheat (Triticum aestivum) L. cv. Jensen sown in autumn 2016, and winter oilseed rape (Brassica napus) L. cv. Explicit) sown in autumn 2017. In the current study, two field experiments were established in 2018 and 2019, respectively, as a two factorial split-plot design block with two factors: soil fertility level (main plot) and cover crop species. Six different species of cover crops were sown as single species (three of them in 2018 and all six in 2019) or in mixtures of two (legume with non-legume): crimson clover (Trifolium incarnatum), buckwheat (Fagopyrum esculetum), hairy vetch (Vicia villosa), oilseed radish (Raphanus sativus), winter rye (Secale cereale) and white lupin (Lupinus alba). Cover crops were sown at 2 cm depth at two soil fertility levels in 3 x 12 m cover crop plots. All treatments were randomized within each of the 4 replicated blocks at each soil fertility level (see below). Detailed information about cover crop species, cultivars and seeding rate are provided in the supplementary materials (Table S1). Seeds of hairy vetch and white lupin were inoculated with Rhizobium (5 ml inoculant suspension kg⁻¹ seeds) a few hours before seeding.

Experiment 1 (Exp. 1) was established on 17 August 2018 and comprised 10 different treatments: control (without cover crops), white lupin, hairy vetch, crimson clover, white lupin + buckwheat, white lupin + winter rye, white lupin + oilseed radish, hairy vetch + buckwheat, hairy vetch + winter rye and hairy vetch + oilseed radish. These treatments were established at two soil fertility levels of the LNTNDT field trial: (1) low fertility level (N₁:2; receiving 60 kg mineral N ha⁻¹ y⁻¹ only and no P or K for more than 50 years) and (2) moderate fertility level (M₁: receiving on average 120 kg NH₄-N ha⁻¹ y⁻¹ in animal slurry for the past 20 years) (van der Bom et al., 2018) corresponding to the common application rate for animal slurry in Danish agriculture.

Experiment 2 (Exp. 2) was established on 20 August 2019 and comprised 13 different treatments. In addition to the same 10 treatments as in Exp. 1, three extra treatments were established: buckwheat, winter
rye and oilseed radish as single cover crops. These treatments were again established at two fertility levels of the LTNDT field trial (but different from those in Exp. 1): (1) low fertility level (N<sub>0</sub>, K<sub>0</sub>; receiving 60 kg mineral N and 60 kg K ha<sup>-1</sup> for the past 20 years) and (2) moderate fertility level (M<sub>P</sub><sub>1</sub>; receiving on average 120 kg NH<sub>4</sub>N ha<sup>-1</sup> y<sup>-1</sup> in animal slurry for the past 20 years and additionally 20 kg mineral P ha<sup>-1</sup> y<sup>-1</sup> for the past 10 years) (van der Bom et al., 2017). Selected soil properties of the chosen soil fertility levels are listed in Table 1.

The control plots were harrowed once in autumn in order to control weed growth, which resulted in more or less bare soil control plots. In Exp. 1, buckwheat and lupin were killed by frost in late autumn and oilseed radish in winter due to low temperatures. The other cover crop species were terminated by soil incorporation on 28 February, well ahead (5 weeks) of spring barley sowing. In Exp. 2, autumn 2019 was dominated by rainy weather followed by a milder winter compared with Exp. 1. Oilseed radish survived the winter. Due to high precipitation in February, soil conditions remained wet throughout March and the cover crops were incorporated on 4 April 2020, just one day prior to the sowing of spring barley. After seedbed harrowing, the subsequent spring barley (cv. Feedway) was sown at a seeding rate of 160 kg ha<sup>-1</sup> on 6 April 2019 in Exp. 1 and on 5 April 2020 in Exp. 2. In Exp. 1, spring barley was fertilized according to the fertility level practice in the LTNDT field trial with 60 kg mineral N and 60 kg K ha<sup>-1</sup> at the low fertility level and 60 kg mineral N ha<sup>-1</sup> and 10 kg P and 73 kg K ha<sup>-1</sup> at the moderate fertility level. In Exp. 2, spring barley was fertilized with 60 kg mineral N ha<sup>-1</sup> and 60 kg K ha<sup>-1</sup> at the low fertility level and 60 kg mineral N ha<sup>-1</sup> and 20 kg mineral P ha<sup>-1</sup> at the moderate fertility level. All field operations were managed in accordance with common Danish farming practice.

Daily weather data were obtained from a climate station close to the field trial. The monthly cumulative precipitation and average temperatures during the experimental period are reported in Fig. 1. The temperature sum was calculated as cumulated growing degree days from sowing to the 2<sup>nd</sup> cut of cover crops by using base temperature of 0 °C. The temperature sum was 992 growing degree days in Exp. 1 and 933 growing degree days in Exp. 2.

### 2.2. Plant sampling, analysis and harvest

The cover crop shoot biomass was sampled twice in the autumn in Exp. 1 (1<sup>st</sup> cut on 27 September and 2<sup>nd</sup> cut on 29 October 2018) and in Exp. 2 (1<sup>st</sup> cut on 1 October and 2<sup>nd</sup> cut on 28 October 2019). Buckwheat was killed by frost before the 2<sup>nd</sup> cut in Exp. 2. Samples were taken from each plot by cutting the biomass at the soil surface in two 0.25 m<sup>2</sup> subplots. The biomass of the mixtures were separated to individual species. The biomass was dried at 60 °C for 48 h and biomass dry matter was determined. For both experimental years, nutrient content was analyzed in the cover crop biomass sampled at 2<sup>nd</sup> cut. At least 10 plants from each species were sampled randomly from each plot, and washed and dried at 60 °C for 48 h prior to analysis. To estimate the content of micro- and macronutrients, the dry shoot biomass was milled and subsequently digested with 2.5 ml 70 % HNO<sub>3</sub> and 1 ml 15 % H<sub>2</sub>O<sub>2</sub>. Shoots were analyzed for their content of P, K, S, Mg and Mn by ICP-OES (Agilent 5100, Agilent Technology, Australia). Total C and N content was analyzed using an elemental CN analyzer (CNS Vario Macro cube, Elementar, Germany). In Exp. 1, there were no Mn values in the ICP analysis due to analytical difficulties. In Exp. 2, the nutrient content in buckwheat was analyzed in the biomass from the 1<sup>st</sup> cut since the buckwheat was killed by frost between the 1<sup>st</sup> and 2<sup>nd</sup> cut.

Spring barley was harvested on 9 August 2019 in Exp. 1 and on 10 August 2020 in Exp. 2. For grain yield measurement, a total area of 15 m<sup>2</sup> (1.5 × 10 m) was harvested at maturity in each experimental plot using an experimental plot combine, recording grain and straw yield. The grain was oven-dried at 60 °C for 48 h and rinsed for weed seeds (constituting on average 2 % of dry matter) and grain dry matter was determined. The N concentration of the grain was analyzed using an elemental CN analyzer (CNS Vario Macro cube, Elementar, Germany). The N yield of barley was calculated by multiplying the N concentration with grain yield.

### 2.3. Calculation of land equivalent ratio (LER)

The Land equivalent ratio was used to assess the growth performance of the cover crop as a single species versus mixtures of two and was calculated based on the shoot biomass sampled in Exp. 2 on 1 and 28 October 2019. LER is defined as the relative land area required to grow a single species to produce the biomass achieved in a mixture (Bedoussac and Justes, 2011). LER for shoot biomass is the sum of the partial LER (LERp) of legume and non-legume species:

\[
LER = LERp(\text{legume}) + LERp(\text{non-legume})
\]

LERp >1 indicates complementarity and over-yielding, where the mixture performance is higher than expected based on the species’ performance when grown as single species. LERp was used to compare the contribution of two species in a mixture and assess to what extent each species benefit from growing in the mixture. LERp > 0.5 indicates that each species performs better in the mixture than as single species, whereas LERp < 0.5 indicates a negative effect.

### 2.4. Statistical analysis

Statistical analysis of the data was performed in R, version 3.1.1 (R Core Team, 2013). The experimental set-up was a split-plot design and the data were analyzed in a linear mixed model from the R-package lme4 with treatments as a fixed effect and block as a random effect. The differences between treatments within each year were analyzed using pairwise comparisons emmeans (estimated marginal means) from the R-package lme4 (Lenth et al., 2018). P values for differences between treatments were adjusted according to the Tukey method. All differences at p ≤ 0.05 were reported as significant. Prior to analysis, data were tested for homogeneity of variance and normality of residuals, and log transformed where necessary (R code is available as supplementary material).

### 3. Results

#### 3.1. Cover crop biomass production and effect of soil fertility level

Growing conditions for cover crops were optimal in Exp. 1 with 130 mm rain in August 2018 followed by a mild and sunny autumn (Fig. 1). It should also be noted that Exp. 1 was preceded by an exceptionally dry spring and summer season, resulting in poor main crop yields and thus presumably leaving elevated levels of nutrients in the soil, making growing conditions even more favourable.

Shoot biomass production of the cover crops was higher in Exp. 1 than in Exp. 2, ranging from 600 to 4000 kg DM ha<sup>-1</sup> in Exp. 1 and from 220 to 2400 kg DM ha<sup>-1</sup> in Exp. 2 at the 2<sup>nd</sup> cut (Figs. 2B, 3B). The effect of soil fertility level on cover crop growth varied greatly between cover

### Table 1

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Soil fertility level</th>
<th>Treatment</th>
<th>P&lt;sub&gt;0.05&lt;/sub&gt;</th>
<th>K&lt;sub&gt;0.05&lt;/sub&gt;</th>
<th>M&lt;sub&gt;P0.05&lt;/sub&gt;</th>
<th>C&lt;sub&gt;0.05&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exp. 1</td>
<td>Low</td>
<td>N&lt;sub&gt;0&lt;/sub&gt;, K&lt;sub&gt;0&lt;/sub&gt;</td>
<td>6.3</td>
<td>61</td>
<td>68</td>
<td>11.2</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>M&lt;sub&gt;1&lt;/sub&gt;</td>
<td>15.3</td>
<td>116</td>
<td>90</td>
<td>14.5</td>
</tr>
<tr>
<td>Exp. 2</td>
<td>Low</td>
<td>N&lt;sub&gt;0&lt;/sub&gt;, K&lt;sub&gt;0&lt;/sub&gt;</td>
<td>5.5</td>
<td>956</td>
<td>62</td>
<td>11.1</td>
</tr>
<tr>
<td></td>
<td>Moderate</td>
<td>M&lt;sub&gt;P1&lt;/sub&gt;</td>
<td>17.0</td>
<td>120</td>
<td>90</td>
<td>13.9</td>
</tr>
</tbody>
</table>
crop species, and was more pronounced in Exp. 2 than in Exp. 1. In both
experiments at 2nd cut, the effect of cover crop species and soil fertility
level on the biomass production of legumes, non-legumes and the total
biomass was significant, except for the effect of soil fertility level on the
growth of non-legumes in Exp. 1 (Figs. 2, 3, Table S2).

Among the legumes, clover and vetch produced less biomass at low
fertility than at moderate fertility level in both years, whereas fertility
level did not affect the growth of lupin. Clover achieved a lower biomass
than lupin and vetch. Within non-legumes in Exp. 1, buckwheat was
least affected by fertility level (Fig. 2). In Exp. 2, all non-legumes had
reduced growth at the low fertility level at some point (Fig. 3). Gener-
ally, the biomass of radish and buckwheat was higher than that of rye,
especially in Exp. 2. In Exp. 1, rye dominated over vetch in the mixture,
whereas in Exp. 2 vetch was the dominant species (Figs. 2, 3).

### 3.2. Synergetic effect of cover crop mixtures

The synergetic effect of cover crop mixtures varied with cover crop
species and soil fertility levels. Legumes produced significantly more
biomass in the mixtures at low fertility than at moderate fertility level,
whereas non-legumes in most cases tended to produce more biomass at
moderate fertility level than at the low soil fertility level (Fig. 4). The
increased share of legume biomass at the low fertility level was even
more pronounced at the 2nd cut. At the 1st cut, none of the mixtures
revealed a significant over-yielding effect, while at the 2nd cut, a non-
significant tendency for over-yielding was observed for the mixtures at
the low fertility level. Lupin + radish, lupin + rye and especially
vetch + radish at the low fertility level achieved LER > 1 (mean LER
= 1.43, 1.31 and 1.47 respectively), indicating the beneficial effect
of these mixtures compared with single species (Fig. 4B). However,
lupin + rye at the moderate fertility level achieved the lowest LER (mean
LER = 0.79) among the mixtures. Non-legume species, buckwheat at 1st
Fig. 3. Shoot biomass of cover crops in Experiment 2 at low and moderate soil fertility levels measured at (A) 1st cut (01 Oct 2019) and (B) 2nd cut (28 Oct 2019). Please note the different scales of the y-axis in the A) and B) plots. Buckwheat was killed by frost before 2nd cut. Presented values are means ± standard error (n = 4). L = legumes, NL = non-legumes, T = total (legumes + non-legumes). Asterisks indicate a significant difference between soil fertility levels within each cover crop species (p < 0.05), n.s. = non-significant.

Fig. 4. Partial land equivalent ratios (LERp) for non-legumes and legumes based on cover crop shoot biomass grown at low (open symbols) and moderate (black symbols) soil fertility levels as measured at A) 1st cut and B) 2nd cut (01 Oct 2019 and 28 Oct 2019). The solid line represents total LER = 1, all treatments above this line indicate complementarity of the mixture. The dashed lines represent LERp of legumes = 0.5 and LERp of non-legumes = 0.5. At the 2nd cut, treatments with buckwheat are missing as buckwheat was killed by frost.
cut and radish at 2nd cut, at the moderate fertility level significantly benefited from growing in mixtures, as the partial LER values were significantly higher than 0.5. At the 2nd cut, lupin was significantly disadvantaged in the mixtures at the moderate fertility with low partial LERs of around 0.19–0.33, whereas it tended to benefit in mixtures at low fertility with partial LERs of around 0.79–0.86 (Fig. 4B). The only mixture that was not affected by soil fertility and did not change from 1st to 2nd cuts was vetch + rye.

3.3. Nutrient content and quality of cover crops

In both experiments, cover crop species and soil fertility level had a significant effect on total shoot N (Figs. 5, 6) at the end of October when maximum biomass and nutrient accumulation are usually observed. In Exp. 1, shoot N content in cover crops ranged from 27 kg N ha\(^{-1}\) for clover at low fertility to 136 kg N ha\(^{-1}\) for vetch + radish at moderate fertility (Fig. 5). While shoot N of clover, vetch and mixtures with vetch was significantly higher at moderate fertility than at low fertility, lupin and its mixtures were not affected. Within legumes, lupin achieved the highest shoot N content, whereas shoot N in radish was the highest among non-legumes. Within mixtures, lupin + radish, vetch + radish and vetch + rye were highest at moderate fertility. At low fertility, partial LERs of lupin + radish, lupin + rye and vetch + radish were highest (Fig. 5).

In Exp. 2, shoot N was lower than the previous year and ranged from 11 kg N ha\(^{-1}\) for rye at low fertility to 86 kg N ha\(^{-1}\) for vetch at moderate fertility (Fig. 6). All single species and mixtures achieved higher shoot N at moderate fertility except for mixtures with lupin, which did not differ between soil fertility levels. For single species, vetch had the highest N content at moderate fertility within legumes, while radish had the highest content among non-legumes. Within mixtures, vetch + radish and lupin + radish had the highest shoot N at both fertility levels.

In both experiments, the shoot content of P, K, S, Mg and Mn varied significantly among cover crop species and soil fertility levels, except for S and Mg in Exp. 1 where the soil fertility level had no effect (Tables 2 and 3). The shoot nutrient content was higher in Exp. 1 than in Exp. 2. Generally, in Exp. 1, mixtures with radish achieved the highest shoot content of K and S, whereas mixtures with buckwheat achieved the highest content of P and Mg. In Exp. 2, the highest content of P, K, S and Mg was reached by radish and mixtures with radish. The shoot P content was higher at moderate fertility than at low fertility, and ranged from 0.9 kg P ha\(^{-1}\) for rye at low fertility (Exp. 2) to 17.3 kg P ha\(^{-1}\) for vetch + buckwheat at moderate fertility (Exp. 1). In most cases, the shoot K content was higher at the moderate fertility level than at the low soil fertility level, ranging from 9.9 kg K ha\(^{-1}\) for rye at low fertility (Exp. 2) to 133 kg K ha\(^{-1}\) for lupin + radish at moderate soil fertility (Exp. 1). The shoot S ranged from 0.9 kg S ha\(^{-1}\) for rye at low fertility (Exp. 2) to 17.2 kg S ha\(^{-1}\) for lupin + radish at low fertility (Exp. 1). The shoot Mg ranged from 0.4 kg Mg ha\(^{-1}\) for rye at low fertility (Exp. 2) to 11.2 kg Mg ha\(^{-1}\) for vetch + buckwheat at low fertility (Exp. 1). The highest values for shoot Mn measured in Exp. 2 were achieved by lupin and mixtures with lupin, resulting in 2–3 kg Mn ha\(^{-1}\).

The C:N, C:P and C:S ratios of shoot biomass were generally lower in Exp. 2 than in Exp. 1 (Table 4). The C:N ratio varied significantly among cover crop species and soil fertility level in both experiments. Buckwheat had the highest C:N ratio (approx. 23 in Exp. 1, but only 15 in Exp. 2 as the C:N ratio was measured at 1st cut due to frost) and vetch had the lowest C:N ratio within a range of 8–9. The C:P ratio varied significantly between cover crop species and soil fertility level in Exp. 2, but not in Exp. 1. Apart from lupin, all species had a C:P ratio below 100 at moderate fertility in Exp. 2, whereas all species in Exp. 1 at both fertility levels and Exp. 2 at low fertility level had a C:P ratio above 100. The C:S ratio was significantly affected by cover crop species in both experiments and by soil fertility level in Exp. 2, where the C:S ratio was higher at the moderate fertility level than at the low fertility level.

3.4. Effect of soil fertility level and cover crops on subsequent barley yield

The grain and N yield of the subsequent spring barley was significantly affected by both cover crop species and soil fertility level in both experiments (Figs. 7, 8, Table S2). In addition, a significant interaction effect on grain yield in Exp. 1 was observed, where mixtures including rye and buckwheat increased the yield compared with the control at the moderate fertility level, but not at the low soil fertility level. The grain yields were higher in Exp. 1 than in Exp. 2, as in Exp. 1 the control achieved grain yields of 3.5 and 5.1 Mg DM ha\(^{-1}\) at the low and moderate fertility level, respectively, whereas in Exp. 2 grain yields were 2.8 and 4.6 Mg DM ha\(^{-1}\), respectively.

In Exp. 1, only vetch significantly increased the grain yield of barley by 30% at low fertility, while at moderate fertility, treatments with...
Table 2

<table>
<thead>
<tr>
<th>Shoot content kg ha⁻¹</th>
<th>P</th>
<th>K</th>
<th>S</th>
<th>Mg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Low soil fertility level</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clover</td>
<td>2.4 ± 0.1 e</td>
<td>17.0 ± 0.9 d</td>
<td>1.5 ± 0.1 e</td>
<td>0.8 ± 0.0 d</td>
</tr>
<tr>
<td>Lupin</td>
<td>7.6 ± 0.3 bc</td>
<td>71.5 ± 2.7 bc</td>
<td>12.4 ± 0.5 b</td>
<td>5.3 ± 0.2 bc</td>
</tr>
<tr>
<td>Vetch</td>
<td>4.5 ± 0.0 de</td>
<td>28.0 ± 0.2 d</td>
<td>3.6 ± 0.1 de</td>
<td>4.2 ± 0.0 c</td>
</tr>
<tr>
<td>Lupin + Buckwheat</td>
<td>10.5 ± 0.7 a</td>
<td>72.9 ± 3.9 abc</td>
<td>9.6 ± 0.4 bc</td>
<td>9.4 ± 0.7 a</td>
</tr>
<tr>
<td>Lupin + Radish</td>
<td>10.2 ± 0.6 ab</td>
<td>94.6 ± 5.5 a</td>
<td>17.2 ± 1.0 a</td>
<td>6.4 ± 0.3 bc</td>
</tr>
<tr>
<td>Lupin + Rye</td>
<td>9.4 ± 1.0 abc</td>
<td>82.6 ± 8.8 ab</td>
<td>10.7 ± 1.0 bc</td>
<td>5.7 ± 0.6 bc</td>
</tr>
<tr>
<td>Vetch + Buckwheat</td>
<td>11.9 ± 1.2 a</td>
<td>75.9 ± 7.4 abc</td>
<td>9.0 ± 0.9 c</td>
<td>11.2 ± 1.1 a</td>
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<tr>
<td>Vetch + Radish</td>
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<td>90.8 ± 7.4 ab</td>
<td>16.3 ± 1.4 a</td>
<td>6.6 ± 0.4 b</td>
</tr>
<tr>
<td>Vetch + Rye</td>
<td>7.0 ± 0.1 cd</td>
<td>55.2 ± 1.2 c</td>
<td>5.1 ± 0.1 d</td>
<td>4.2 ± 0.1 c</td>
</tr>
<tr>
<td><strong>Moderate soil fertility level</strong></td>
<td></td>
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</tr>
<tr>
<td>Clover</td>
<td>3.5 ± 0.5 d</td>
<td>33.9 ± 4.7 d</td>
<td>3.8 ± 0.5 d</td>
<td>1.7 ± 0.2 d</td>
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<tr>
<td>Lupin</td>
<td>11.2 ± 0.7 bc</td>
<td>113.2 ± 7.2 ab</td>
<td>15.6 ± 1.0 a</td>
<td>4.1 ± 0.3 c</td>
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<td>Vetch</td>
<td>8.0 ± 0.5 c</td>
<td>37.2 ± 2.5 d</td>
<td>3.3 ± 0.2 d</td>
<td>4.0 ± 0.3 cd</td>
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<tr>
<td>Lupin + Buckwheat</td>
<td>14.6 ± 1.5 ab</td>
<td>102.4 ± 9.6 abc</td>
<td>9.6 ± 0.8 c</td>
<td>7.4 ± 0.8 ab</td>
</tr>
<tr>
<td>Lupin + Radish</td>
<td>13.4 ± 1.4 ab</td>
<td>133.7 ± 1.4 a</td>
<td>15.0 ± 1.4 ab</td>
<td>6.9 ± 0.9 ab</td>
</tr>
<tr>
<td>Lupin + Rye</td>
<td>9.2 ± 0.4 c</td>
<td>79.0 ± 3.8 bc</td>
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<td>4.6 ± 0.3 c</td>
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<tr>
<td>Vetch + Buckwheat</td>
<td>17.3 ± 0.7</td>
<td>109.2 ± 4.9 ab</td>
<td>9.6 ± 0.4 c</td>
<td>8.9 ± 0.4 a</td>
</tr>
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<td>Vetch + Radish</td>
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<td>7.1 ± 0.4 a</td>
</tr>
<tr>
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<td>67.9 ± 7.0 cd</td>
<td>8.5 ± 0.9 c</td>
<td>6.1 ± 0.6 bc</td>
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<td></td>
<td></td>
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<tr>
<td>Cover crop (CC)</td>
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<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
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<tr>
<td>Soil fertility (SF)</td>
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<td>&lt; 0.001</td>
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<td>n.s.</td>
</tr>
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Table 3

<table>
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<tr>
<th>Shoot content kg ha⁻¹</th>
<th>P</th>
<th>K</th>
<th>S</th>
<th>Mg</th>
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<tr>
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<tr>
<td>Buckwheat</td>
<td>2.9 ± 0.3 bc</td>
<td>31.4 ± 3.1 b</td>
<td>1.9 ± 0.2 b</td>
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</tr>
<tr>
<td>Radish</td>
<td>4.1 ± 0.3 ab</td>
<td>64.6 ± 4.3 a</td>
<td>9.5 ± 0.6 a</td>
<td>2.1 ± 0.1 cde</td>
</tr>
<tr>
<td>Rye</td>
<td>0.9 ± 0.2 d</td>
<td>9.9 ± 2.3 b</td>
<td>0.9 ± 0.2 b</td>
<td>0.4 ± 0.1 c</td>
</tr>
<tr>
<td>Clover</td>
<td>1.0 ± 0.2 cd</td>
<td>10.1 ± 2.0 b</td>
<td>1.0 ± 0.2 b</td>
<td>0.7 ± 0.1 ef</td>
</tr>
<tr>
<td>Lupin</td>
<td>2.1 ± 0.4 cd</td>
<td>26.2 ± 5.5 ab</td>
<td>1.9 ± 0.4 b</td>
<td>1.4 ± 0.3 cdef</td>
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<tr>
<td>Vetch</td>
<td>3.0 ± 0.3 bc</td>
<td>21.8 ± 2.2 b</td>
<td>2.1 ± 0.2 b</td>
<td>1.6 ± 0.2 bcde</td>
</tr>
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<td>Lupin + Radish</td>
<td>4.1 ± 0.6 ab</td>
<td>60.0 ± 8.9 ab</td>
<td>7.5 ± 1.3 a</td>
<td>2.3 ± 0.3 abc</td>
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<td>Lupin + Rye</td>
<td>2.1 ± 0.3 cd</td>
<td>25.6 ± 4.3 b</td>
<td>1.9 ± 0.3 a</td>
<td>1.3 ± 0.2 cdef</td>
</tr>
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<td>Vetch + Radish</td>
<td>5.0 ± 1.3 a</td>
<td>58.5 ± 15 a</td>
<td>7.7 ± 1.9 a</td>
<td>2.6 ± 0.7 ab</td>
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<td>Vetch + Rye</td>
<td>2.1 ± 0.2 cd</td>
<td>16.8 ± 1.9 b</td>
<td>1.6 ± 0.2 b</td>
<td>1.1 ± 0.1 def</td>
</tr>
<tr>
<td><strong>Moderate soil fertility level</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Buckwheat</td>
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<td>78 ± 3.5 b</td>
<td>3.4 ± 0.2 bc</td>
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</tr>
<tr>
<td>Radish</td>
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<td>127.2 ± 9.8 a</td>
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<td>4.1 ± 0.3 ab</td>
</tr>
<tr>
<td>Rye</td>
<td>2.6 ± 0.3 e</td>
<td>25.7 ± 2.8 de</td>
<td>1.9 ± 0.2 bc</td>
<td>1.1 ± 0.1 ef</td>
</tr>
<tr>
<td>Clover</td>
<td>3.9 ± 0.5 de</td>
<td>31.1 ± 4.3 cde</td>
<td>2.3 ± 0.3 bc</td>
<td>2.2 ± 0.3 de</td>
</tr>
<tr>
<td>Lupin</td>
<td>4.7 ± 0.4 de</td>
<td>39.6 ± 3.5 cde</td>
<td>2.9 ± 0.3 bc</td>
<td>2.5 ± 0.2 cd</td>
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<td>Vetch</td>
<td>9.1 ± 1.0 bc</td>
<td>57.2 ± 6.4 bc</td>
<td>4.6 ± 0.5 b</td>
<td>3.6 ± 0.4 bc</td>
</tr>
<tr>
<td>Lupin + Radish</td>
<td>10.7 ± 0.4 ab</td>
<td>109.9 ± 4.3 a</td>
<td>11.1 ± 0.4 a</td>
<td>3.8 ± 0.2 cb</td>
</tr>
<tr>
<td>Lupin + Rye</td>
<td>2.6 ± 0.5 e</td>
<td>23.7 ± 4.8 de</td>
<td>1.8 ± 0.4 c</td>
<td>1.2 ± 0.2 ef</td>
</tr>
<tr>
<td>Vetch + Radish</td>
<td>13.0 ± 1.5 a</td>
<td>125.6 ± 16 a</td>
<td>12.6 ± 1.6 a</td>
<td>4.5 ± 0.5 ab</td>
</tr>
<tr>
<td>Vetch + Rye</td>
<td>6.4 ± 0.9 cd</td>
<td>44.0 ± 6.5 cd</td>
<td>3.5 ± 0.5 bc</td>
<td>2.6 ± 0.4 cd</td>
</tr>
<tr>
<td><strong>Analysis of variance (ANOVA) p-value</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cover crop (CC)</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Soil fertility (SF)</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>CC x SF</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

clover, vetch, lupin + radish, vetch + buckwheat and vetch + radish increased the yield by 13–19 % (Fig. 7A). The N yield was also significantly increased by several treatments, mostly by vetch, which resulted in a 40 % (20 kg N ha⁻¹) and 48 % (33 kg N ha⁻¹) increase at low and moderate soil fertility levels respectively (Fig. 7B). In Exp. 2, the grain yield was significantly increased by 46 % by vetch + radish, whereas rye significantly decreased the yield by 43 % at the low soil fertility level (Fig. 8A). While there was a tendency for a
positive effect on yield by several treatments at the low fertility level, the
grain yield in all treatments with lupin was the same as the control. At
the moderate fertility level, all cover crop treatments increased the
yield, but only significantly so in the vetch + radish treatment. At low
fertility, the treatment with vetch + radish increased N yield significantly
by 44 %, corresponding to 22 kg N ha$^{-1}$ (Fig. 8B). At moderate fertility,
the N yield increased significantly by 19–38 % in treatments with vetch,
Vetch + radish and all mixtures with vetch, corresponding to 14–28 kg N
ha$^{-1}$.

Grain harvested in Exp. 2 was also analyzed for the content of P, K, S,
Mg and Mn (Table 5). The grain nutrient content was significantly
affected by both cover crop species and soil fertility level, especially
to low fertility. Generally at low fertility, rye significantly decreased the P
content in grain compared with the control, and showed the same tend-
cy for K, S, Mg and Mn. Vetch + radish significantly increased the
content of S and Mn at low fertility and the content of S at moderate
fertility compared with the control.

When looking at grain nutrient concentrations (Table S6), the N, P, S,
Mg and Mn concentrations were all affected by soil fertility level, being
higher for P, S and Mg at the moderate soil fertility level than at the low
soil fertility level, while it was the opposite for Mn. Only N and S concen-
trations were significantly affected by cover crop species, with vetch
(single or in mixtures) generally inducing the highest concentrations of
both.

4. Discussion

4.1. Effect of soil fertility level and weather conditions on cover crop
biomass production and nutrient acquisition

Adequate biomass production and nutrient contents of cover crops
are essential for their potential positive effect on the subsequent main
crop. Biomass production and nutrient contents of the cover crops in
spring were higher in Exp. 1 (up to 4000 kg DM ha$^{-1}$ and 140 kg N
ha$^{-1}$, Fig. 2) than in Exp. 2 (up to 2500 kg DM ha$^{-1}$ 80 kg N ha$^{-1}$,
Fig. 3), probably due to more favorable weather conditions in Exp. 1.
hairy vetch produced less biomass and had a lower shoot N content at the low fertility level than at the moderate fertility level in both experiments. White lupin was not able to mobilize P in soil by releasing protons (Kreuzeder et al., 2018). Besides biomass production, nutrient acquisition in cover crops is an important trait for improving nutrient cycling in agroecosystems. However, only a few studies have quantified the content of nutrients other than N and in a few cases S (Wendling et al., 2016). In the present study, cover crops accumulated up to 140 kg N ha\(^{-1}\), but also 17 kg P ha\(^{-1}\), 133 kg K ha\(^{-1}\), 17 kg S ha\(^{-1}\) and 11 kg Mg ha\(^{-1}\). The nutrient content of cover crops measured in late autumn, at the peak of biomass growth, varied considerably between species and was also affected by soil fertility level. The nutrient content was significantly higher at the moderate fertility level than at the low fertility level in both experiments for P and K, and in Exp. 2 also for S and Mg. It is noteworthy that the C:N, C:P and C:S ratios were lower in Exp. 2, which can be attributed to the poorer weather conditions that autumn. Thus, favourable growing conditions in Exp. 1 caused a dilution effect on nutrient concentrations, leading to higher C-to-nutrient ratios, which in turn may result in more nutrient immobilization after soil incorporation of the cover crop residues, and reduced nutrient availability for the subsequent crop (discussed further in Section 4.3). However, when assessing the nutrient concentrations (Tables S3 and S4) measured in the cover crops in late October in both experiments, there was no indication that a single nutrient was limiting.

Repeated application of organic fertilizer (cattle or pig slurry) for more than 20 years at the moderate soil fertility level has led to higher soil organic carbon levels (Table 1) and residual effects of N, P and K than at the low fertility level with mineral amendments only. Moreover, application of organic fertilizers can lead to improved soil physical properties (Peltre et al., 2015). From visual observations in the field, it is clear that the soil structure in the low fertility level plots is poorer and that the surface tends to seal after tillage and rain. The significant differences found in nutrient content in cover crops at the two soil fertility levels (N, P, K and in the Exp. 2 also S and Mg) may therefore be due to a synergy between nutrient availability in autumn and soil organic carbon levels affecting soil physical conditions and hence cover crop growth.

### 4.2. Effect of soil fertility level on over-yielding in cover crop mixtures

The goal of growing legume and non-legume species together is to increase the benefit of each species due to their complementarity and to thereby improve the performance of the mixture. The results of this study show that interactions in cover crop mixtures can be both positive and negative, depending on the cover crop species and soil fertility level. Legumes produced more biomass than non-legumes in the mixtures at the low fertility level, whereas non-legumes in most cases tended to
soil fertility is in line with previous studies (Wortman et al., 2012; Tri)

to provide multiple benefits, e.g. both N scavenging and N fixation. This

and radish dominated the mixtures and strongly limited the biomass

with other studies (Tribouillois et al., 2016; Wendling et al., 2017).

produce more biomass at the moderate fertility level, which is consistent

with other studies (Tribouillois et al., 2016; Wendling et al., 2017).

The partial LER show that at the moderate fertility level, buckwheat

and radish dominated the mixtures and strongly limited the biomass

production of the legumes and thereby also the ability of these mixtures

to provide multiple benefits, e.g. both N scavenging and N fixation. This

interspecific competition of fast-growing non-legume species at higher

soil fertility is in line with previous studies (Wortman et al., 2012; Tri-

bouillois et al., 2016), but its effects depend on the competitive ability

of the non-legume species. In the present study, winter rye was less

competitive than radish and buckwheat, and thus did not dominate the

mixtures due to its lower growth. Despite the low competitive ability of

rye, lupin+rye resulted in a negative interaction, indicating low

complementarity between these two species at the moderate fertility

level.

Meanwhile, at the low fertility level, legumes dominated the mix-
tures because they can access atmospheric N through biological fixation

and are not limited by soil N availability. This tendency increased from

1st to 2nd cuts and resulted in over-yielding of the mixtures lupin+radish,
lupin+rye and vetch+radish. However, radish showed over-yielding at

low fertility as well, which also contributed to over-yielding of its

mixtures, highlighting the complementarity of these species at low

fertility.

The relative proportion of each species in the mixture can also in-
fluence the biomass production and complementarity of the species

(Hayden et al., 2014; Wendling et al., 2017). Thus, finding the right

mixture seeding rate for the specific soil fertility status is important in

order to improve the performance of the mixtures under variable condi-
tions. Moreover, great difference in root morphological and physio-

logical traits among cover crops demonstrates a potential for functional

diversity benefits but also a need for more research in order to design

cover crop mixtures providing positive complementarity for root growth

and nutrient acquisition (Wendling et al., 2016; Honvault et al., 2021).

4.3. Effect of soil fertility level and cover crop performance on grain and

Yield of succeeding barley crop

In both experiments, spring barley yields were substantially affected

by soil fertility levels (p < 0.001, Table S2). Across the cover crop

treatments, the yield increase at moderate fertility amounted to 48% in

Exp. 1 and 64% in Exp. 2 compared with the low fertility level. This is

in line with previous observations from the Long-Term Nutrient Deple-
tion Trial (van der Bom et al., 2017). Grain N concentrations were quite

low, on average 1.7% across treatments, indicating that yields were N

limited at both fertility levels (Table S6). The exact cause of the much

lower yield at the low fertility level remains elusive, but tillering of

barley was observed to be depressed at the low fertility level. It may be

speculated that the differences we observed between fertility levels is

the result of differences in nutrient pools and fluxes, plant-soil-microbe

relations, decomposition dynamics and soil structure occurring in the

long-term treatments. As discussed above, poor soil structure and low

availability of soil P have previously been shown to cause substantial

yield depressions (van der Bom et al., 2017), especially during the early

stage of spring barley development. The current paper can perhaps be

seen as a documentation of how large the resulting effects are of these

processes at different fertility levels.

Cover crops had greater potential to increase grain and N yields at

moderate than at low soil fertility levels. This could be due to a sub-

stantially higher cover crop uptake of N, P, K and for Exp. 2 also S and

Mg at the moderate fertility level. However, yield improvements also

<table>
<thead>
<tr>
<th>Grain content</th>
<th>P kg ha⁻¹</th>
<th>K kg ha⁻¹</th>
<th>S kg ha⁻¹</th>
<th>Mg kg ha⁻¹</th>
<th>Mn g ha⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low soil fertility level</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>9.1 ± 1.5 a</td>
<td>15.2 ± 2.7 ab</td>
<td>3.1 ± 0.5 bc</td>
<td>2.7 ± 0.4 ab</td>
<td>26.5 ± 4.8 bc</td>
</tr>
<tr>
<td>Buckwheat</td>
<td>11.8 ± 0.9 a</td>
<td>18.8 ± 1.2 a</td>
<td>3.9 ± 0.2 ab</td>
<td>3.5 ± 0.2 a</td>
<td>32.6 ± 2.6 ab</td>
</tr>
<tr>
<td>Radish</td>
<td>10.5 ± 1.5 a</td>
<td>18.5 ± 2.9 a</td>
<td>4.0 ± 0.6 ab</td>
<td>3.3 ± 0.5 a</td>
<td>35.7 ± 6.3 ab</td>
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<td>Rye</td>
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<td>81.1 ± 2.4 b</td>
<td>1.8 ± 0.5 ab</td>
<td>1.4 ± 0.4 b</td>
<td>14.8 ± 4.0 c</td>
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<tr>
<td>Clover</td>
<td>10.6 ± 1.8 a</td>
<td>19.2 ± 3.9 a</td>
<td>4.2 ± 0.8 ab</td>
<td>3.4 ± 0.7 a</td>
<td>37.8 ± 5.4 ab</td>
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<tr>
<td>Lupin</td>
<td>8.7 ± 1.4 ab</td>
<td>15.3 ± 2.6 ab</td>
<td>3.2 ± 0.5 bc</td>
<td>2.7 ± 0.5 a</td>
<td>30.8 ± 5.5 ab</td>
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<tr>
<td>Vetch</td>
<td>10.8 ± 1.7 a</td>
<td>19.0 ± 3.2 a</td>
<td>4.3 ± 0.7 ab</td>
<td>3.4 ± 0.6 a</td>
<td>39.4 ± 7.5 ab</td>
</tr>
<tr>
<td>Lupin + Buckwheat</td>
<td>9.1 ± 2.1 a</td>
<td>15.1 ± 3.4 ab</td>
<td>3.2 ± 0.7 bc</td>
<td>2.8 ± 0.7 a</td>
<td>28.2 ± 6.4 ab</td>
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<tr>
<td>Lupin + Radish</td>
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<td>3.5 ± 0.8 ab</td>
<td>2.8 ± 0.6 a</td>
<td>32.5 ± 7.7 ab</td>
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<td>Lupin + Rye</td>
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<td>14.9 ± 1.9 ab</td>
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<td>28.2 ± 3.2 bc</td>
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<td>19.7 ± 2.4 a</td>
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<td>3.5 ± 0.4 a</td>
<td>37.6 ± 3.4 bc</td>
</tr>
<tr>
<td>Vetch + Radish</td>
<td>12.2 ± 1.2 a</td>
<td>21.6 ± 1.7 a</td>
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<td>3.9 ± 0.4 a</td>
<td>42.4 ± 3.1 a</td>
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<tr>
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<td>10.5 ± 0.8 a</td>
<td>18.6 ± 1.2 a</td>
<td>4.2 ± 0.2 ab</td>
<td>3.3 ± 0.2 a</td>
<td>35.8 ± 0.4 ab</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>17.7 ± 1.4 a</td>
<td>25.0 ± 1.9 a</td>
<td>5.4 ± 0.3 b</td>
<td>5.0 ± 0.4 a</td>
<td>37.8 ± 3.2 a</td>
</tr>
<tr>
<td>Buckwheat</td>
<td>20.7 ± 2.1 a</td>
<td>28.8 ± 2.6 a</td>
<td>6.1 ± 0.6 ab</td>
<td>5.8 ± 0.6 a</td>
<td>40.9 ± 7.2 a</td>
</tr>
<tr>
<td>Radish</td>
<td>19.8 ± 0.9 a</td>
<td>28.3 ± 1.5 a</td>
<td>6.1 ± 0.3 ab</td>
<td>5.7 ± 0.3 a</td>
<td>43.4 ± 2.1 a</td>
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<tr>
<td>Rye</td>
<td>20.3 ± 1.8 a</td>
<td>28.3 ± 2.3 a</td>
<td>6.1 ± 0.6 ab</td>
<td>5.5 ± 0.4 a</td>
<td>40.8 ± 3.2 a</td>
</tr>
<tr>
<td>Clover</td>
<td>20.0 ± 0.1 a</td>
<td>27.9 ± 0.8 a</td>
<td>6.1 ± 0.1 ab</td>
<td>5.9 ± 0.1 a</td>
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<tr>
<td>Lupin</td>
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<td>29.2 ± 1.5 a</td>
<td>6.3 ± 0.2 ab</td>
<td>5.8 ± 0.3 a</td>
<td>42.9 ± 3.9 a</td>
</tr>
<tr>
<td>Vetch</td>
<td>20.5 ± 1.3 a</td>
<td>29.2 ± 2.1 a</td>
<td>7.0 ± 0.4 a</td>
<td>6.2 ± 0.3 a</td>
<td>47.7 ± 2.6 a</td>
</tr>
<tr>
<td>Lupin + Buckwheat</td>
<td>19.9 ± 1.1 a</td>
<td>27.6 ± 1.3 a</td>
<td>5.8 ± 0.3 ab</td>
<td>5.5 ± 0.3 a</td>
<td>39.9 ± 3.1 a</td>
</tr>
<tr>
<td>Lupin + Radish</td>
<td>20.3 ± 0.6 a</td>
<td>28.4 ± 0.6 a</td>
<td>6.2 ± 0.1 ab</td>
<td>5.7 ± 0.1 a</td>
<td>42.1 ± 3.4 a</td>
</tr>
<tr>
<td>Lupin + Rye</td>
<td>21.8 ± 2.1 a</td>
<td>29.9 ± 2.6 a</td>
<td>6.5 ± 0.6 ab</td>
<td>6.1 ± 0.6 a</td>
<td>37.9 ± 4.6 a</td>
</tr>
<tr>
<td>Vetch + Buckwheat</td>
<td>21.2 ± 0.7 a</td>
<td>30.0 ± 0.8 a</td>
<td>7.0 ± 0.2 a</td>
<td>6.2 ± 0.2 a</td>
<td>44.9 ± 3.6 a</td>
</tr>
<tr>
<td>Vetch + Radish</td>
<td>21.2 ± 0.5 a</td>
<td>30.4 ± 0.2 a</td>
<td>7.0 ± 0.2 a</td>
<td>6.2 ± 0.1 a</td>
<td>53.4 ± 4.4 a</td>
</tr>
<tr>
<td>Vetch + Rye</td>
<td>20.4 ± 0.7 a</td>
<td>28.6 ± 1.4 a</td>
<td>6.6 ± 0.2 ab</td>
<td>5.9 ± 0.2 a</td>
<td>41.7 ± 3.6 a</td>
</tr>
</tbody>
</table>

Analysis of variance (ANOVA) p-value

| Cover crop (CC) | < 0.01 | < 0.01 | < 0.001 | < 0.001 | < 0.001 |
| Soil fertility (SF) | < 0.001 | < 0.001 | < 0.001 | < 0.001 | n.s. |
| CC x SF | < 0.05 | < 0.05 | n.s. | n.s. | n.s. |
These results indicate that biomass production and the nutrient content of cover crops measured in late autumn are not always related to an increase in yield of the subsequent crop. Furthermore, the cover crops that were least affected by low fertility did not lead to a higher residual effect on the succeeding barley crop. Despite the higher shoot N, P, K and S content in lupin than in vetch in Exp. 1, vetch increased the grain yield at both soil fertility levels whereas lupin did not. Vetch was the only cover crop that significantly increased the yield of the subsequent spring barley, even though the shoot N content of vetch measured in autumn of Exp. 1 was within the same range or even lower than most of the other treatments at low fertility. Furthermore, crimson clover significantly increased the grain and N yield of barley at moderate fertility level in Exp. 1, although it reached the lowest shoot biomass and shoot N, P, K, S and Mg content in autumn. This may be due to a higher shoot N content and soil mineral N content at incorporation time for winter-persistent legumes (hairy vetch and crimson clover) compared with non-persistent legumes (white lupin), as shown in study of Thorup-Kristensen (2006a), and thereby better synchrony between nutrient release from the residues and the nutrient demand of the subsequent crop (Langelier et al., 2021; Li et al., 2015b). Hairy vetch is known to release from the residues and the nutrient demand of the succeeding crop (Langelier et al., 2021) due to typically high N concentrations and therefore low C:N ratios (8–9) just before incorporation, which result in relatively fast N release after incorporation (Hansen et al., 2021). The white lupin used in the present study was killed by frost in the autumn, which could lead to an earlier onset of N mineralization and potentially higher N loss (Thorup-Kristensen, 2006a). Therefore, growing winter-persistent lupin species could perhaps improve the cover crop effect on the subsequent main crop, which in combination with its ability to grow well at low soil fertility levels could make it a very valuable cover crop for low fertility soils. Buckwheat was also killed by frost in the autumn and reached a high C:N ratio, which is most likely why no yield improvements in barley were observed after buckwheat.

The N content of cover crops was only measured in aboveground biomass but not belowground root biomass, which underestimated total cover crop N content; this is particularly important for legumes, where belowground residues can contribute substantially to grain and N yield of the succeeding crop (Li et al., 2015b). The lupin–radish mixture and lupin alone reached a shoot N content of approximately 120 kg N ha⁻¹ in Exp. 1, however, only the mixture increased the barley grain and N yield at the moderate fertility level. This could be because lupin was killed by frost earlier than radish, but also due to the potential N contribution from the root biomass of radish that was not measured in the current study (Li et al., 2015b; Hansen et al., 2021).

Several studies have reported higher effects on grain and N yields after legume cover crops than after non-legumes due to the higher N concentrations and lower C:N ratios (Li et al., 2015a; Valkama et al., 2015). Although the C:N ratios of all cover crops in Exp. 2 measured in March 2020 (just before incorporation and sowing of the spring barley) were below 15 (Table S5), suggesting relatively fast release of N from all cover crop residues after incorporation, the effect on the succeeding crop ranged from negative for rye (C:N 13–14) to positive for vetch–radish (C:N 11–12). In fact, in Exp. 2 only the mixture of radish and vetch increased the yield significantly at both soil fertility levels. Besides a low C:N ratio of vetch (8–9) and low C:S ratio of radish (50–65), this could also be due to positive complementarity of radish and vetch when grown in a mixture, as indicated by the high LER as well as high shoot contents of P, K, S and Mg in this mixture. Oilseed radish has previously shown higher root length density then vetch, which is important for soil exploration and acquisition of nutrients with restricted mobility (Wendling et al., 2016). The ability of high P and S uptake by radish have been observed in previous studies (Eriksen et al., 2004; Couëdel et al., 2018; Hansen et al., 2021). Furthermore, autumn and winter precipitation was higher in Exp. 2 than in Exp. 1, so another reason for the increase in yield could be the ability of deep-rooted radish that survived the winter in Exp. 2 to decrease N and S leaching and thereby maintain greater residual soil N and S for the following barley compared with the control (Eriksen and Thorup-Kristensen, 2002; Thorup-Kristensen, 2006b). The results from the current study demonstrate that growing winter-persistent legumes such as hairy vetch and crimson clover alone or in a mixture with a fast-growing, vigorous non-legume such as oilseed radish is a promising tool to improve yields of the subsequent crop through nitrogen input and nutrient cycling.

These results also indicate that winter rye is not a suitable cover crop for cultivation in soils with a low fertility level. Winter rye was the non-legume with the lowest biomass production and shoot N, P, K, S and Mg content. At low fertility, a decreased spring barley yield was observed after rye incorporation in Exp. 2, which may be due to the depletion of soil N during rye growth just before incorporation (pre-emptive competition), as shown in other studies (Thorup-Kristensen and Dresboll, 2010; Li et al., 2015b). Another reason could be the lack of time for N mineralization, as in Exp. 2 barley was sown only one day after incorporation of rye, whereas in Exp. 1 rye was incorporated five weeks before sowing. Late incorporation allows less time for N mineralization and thus the timing of cover crop incorporation is a crucial factor for N supply for the subsequent crop (Blanco-Canqui et al., 2015). Hence, the results of this study suggest that besides the selection of cover crop species and mixtures, management practices affect the nutrient supply and yield of the subsequent cash crops, especially in a low fertility soil.

5. Conclusions

The results of this two-year field trial demonstrated that the growth and nutrient content of cover crops in autumn were largely influenced by species, mixture choices and soil fertility level. The growth of some species was less affected by soil fertility level than that of others. White lupin was the legume least affected by soil fertility level, whereas crimson clover and hairy vetch showed reduced biomass production and nutrient content at low fertility. Among the non-legumes, buckwheat was the species with the highest biomass production and highest C:N at the low fertility level in both experimental years, and also the species that was least affected by the soil fertility level in Exp. 1. When cover crops were cultivated in mixtures, non-legumes tended to produce more biomass at the moderate than at the low soil fertility level, whereas legumes produced more biomass at low fertility. Higher over-yielding was found in the vetch–radish, lupin–radish and lupin–rye mixtures at low fertility than at moderate, indicating complementarity of these species at low fertility level.

However, the cover crops which growth were least affected by low soil fertility level did not lead to a higher residual effect on the succeeding barley crop at low fertility, probably due to factors such as C-to-nutrient ratios and lack of winter persistency. Vetch grown as a single species and as a mixture with radish produced the greatest improvements in spring barley yield regardless of soil fertility level, whereas rye had a negative effect on grain yields at low fertility. Soil fertility level, nevertheless, had a greater effect on grain yield than the cover crop treatments.
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CRediT authorship contribution statement

Veronika Hansen: Methodology, Investigation, Formal analysis, Visualization, Writing – Original draft, Project administration. Linn V. Meilvang: Investigation, Formal analysis, Writing – Original draft. Jakob Magid: Conceptualization, Methodology, Writing – Review & Editing, Funding acquisition. Kristian Thorup-Kristensen: Conceptualization, Methodology, Writing – Review & Editing, Funding acquisition. Lars Stoumann Jensen: Conceptualization, Methodology, Writing – Review & Editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.eja.2023.126796.

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