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1 Can living mulches in intercropping systems reduce the risk of nitrate leaching: Studies of organic
2 cauliflower (*Brassica oleracea* L.) and leek (*Allium porrum* L.) production across European
3 conditions
4

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1 Abstract:

2 The feasibility of living mulches (LMs) to reduce the risk of nitrate leaching when grown with
3 vegetables with high nitrogen (N) demand and low use efficiency was investigated in four European
4 countries: Italy, Slovenia, Germany, and Denmark over two years. The plant N uptake and soil
5 inorganic N (N_{inorg}) content at harvest, in the autumn, and in the following spring were measured
6 below open-pollinated and hybrid cultivars of cauliflower and leek crops. The cultivars were
7 consistently different over the two years. In Italy and Slovenia, when LM of burr medic or white
8 clover was broad sown (addition design ADD) at the same time as crop transplanting, the N uptake
9 of crops was hampered without increasing the total aboveground N accumulation. Delaying LM
10 sowing by 2.5 to 4 weeks maintained the N uptake of crops. In Germany, delaying white clover
11 sowing for a month in cauliflower reduced soil N_{inorg} at the start of leaching by 17 to 33 kg ha⁻¹ in
12 the ADD design and 25 kg ha⁻¹ in the substitution design (SUB), where rows of cash crops were
13 replaced by rows of LM. An overwintering LM of grass-clover incorporated in strips and root
14 pruning decreased soil N_{inorg} at the start of the leaching season by 33 kg N ha⁻¹ in cauliflower, and
15 in leek, an LM of dyers woad decreased the soil N_{inorg} by 52 kg N ha⁻¹ at harvest and 38 kg N ha⁻¹ at
16 the start of leaching. The effect of delayed LM sowing depended on the LM species and system
17 design. Overall, the SUB and ADD design in LM systems showed potential to reduce the risk of
18 nitrate leaching, whereas the ADD design had stronger competition with either cash crop or LM.
19 The key to reduce soil N_{inorg} without jeopardizing crop yields may be to identify suitable growing
20 periods, sometimes combined with root pruning, for each LM species/design. Overall, these
21 findings indicate that it was possible to use the introduction strategy to control competition and
22 reduce leaching, but the performance depended on the intensity of the interspecific competition and
23 the local conditions.

24 Keywords:

- 1 Nitrate leaching, *Allium porrum* L., *Brassica oleracea* L., *Trifolium repens* L., *Medicago polymorpha*
- 2 L., *Isatis tinctoria* L., living mulch, sowing dates, substitution design

3

1 Introduction

2 Vegetable production has been reported to cause nitrate contamination of groundwater (Babiker et
 3 al., 2004, Song et al., 2009, Benoit et al., 2014) due to the high inputs of N fertilizer and low N use
 4 efficiency. Efficient N management has been applied and shown positive effects in conventional
 5 and organic vegetable production on reducing nitrate leaching, for example, decreased synthetic N
 6 fertilizer rate without yield losses (de Paz et al., 2004), crop rotations with improved N use
 7 efficiency (Vos et al., 2004, Thorup-Kristensen, 2006) and autumn catch crops taking up soil N
 8 after the harvest (Thorup-Kristensen, 1994, Wyland et al., 1996). However, these strategies are
 9 difficult in the case of vegetables such as leek (*Allium porrum* L.) and cauliflower (*Brassica*
 10 *oleracea* L.), which demand high N availability during the growing season when they are: late-
 11 harvested before a cold season without plant production (continental and temperate regions) or
 12 followed immediately by another crop (Mediterranean regions). Therefore, a high amount of N may
 13 be left in the soil and can be leached and lost from the agro-ecosystem before it is taken up by a
 14 succeeding crop that is established right after harvest or the following spring. For example,
 15 cauliflower left 50-80 kg inorganic N (N_{inorg}) ha^{-1} in the soil (0-0.6 m) (Everaarts et al., 1996) and
 16 leached as much as 293 kg $N-NO_3^-$ ha^{-1} below a 0.9 m depth in a conventional field (De Neve et al.,
 17 2003), whereas soil N_{inorg} after leek harvest was 133 kg N ha^{-1} in the 1-2.5 m soil layer (Thorup-
 18 Kristensen, 2006). Therefore, growing a cash crop intercropped with a living mulch (LM) could be
 19 effective to retain N during the cropping cycle and/or the leaching season (i.e., winter), N that
 20 would otherwise be lost by leaching. Living mulches (LMs) were found to decrease the risk of N
 21 leaching in both conventional (Manevski et al., 2015, Mariotti et al., 2015) and organic cereal and
 22 vegetable systems (Whitmore et al., 2007, Thorup-Kristensen et al., 2012). Higher or comparable
 23 yield and quality of cash crop in LM systems was achieved when the interspecific competition was
 24 negligible (Båth et al., 2008) and LM suppressed pests and weeds (Brainard et al., 2004). In other
 25 cases, interspecific competition resulted in high yield losses of crops (Lotz et al., 1997).

1 The choice of LM species is important to the outcome in LM systems. The species should be
 2 selected on the basis of its features, the competitiveness of the cash and LM crops, and the
 3 ecological benefits that the LM is able to provide. The N uptake of the LM varies from species to
 4 species (Campiglia et al., 2010). Legumes are commonly used as LMs in cereal production to
 5 improve cereal yield in low N-input systems, especially in organic systems, due to their biological
 6 nitrogen fixation (BNF) and less competition with cereals (Bedoussac et al., 2010, Bedoussac et al.,
 7 2015). In such systems, less N fertilizer is needed due to the BNF increase of the soil N_{inorg} (Abad et
 8 al., 2004). Compared to legume sole crop, the combination of legume and non-legume depleted
 9 more soil N_{inorg} due to the complimentary use of fixed N and soil N_{inorg} (Hauggaard-Nielsen et al.,
 10 2003). In other cases, plant species from the *Brassica* family have been used as LM due to their
 11 potential for weed suppression without yield reduction (Fernandez et al., 2015) and have been used
 12 as autumn catch crops because of their high N uptake capacity and deep root systems (Thorup-
 13 Kristensen, 2006, Herrera et al., 2010). Furthermore, cultivars of cash crops have shown different
 14 competitiveness against LMs (Picard et al., 2010). Although interspecific competition existed in
 15 several cases, it was possible to control or alleviate it by management strategies. For example, root
 16 pruning increased the aboveground biomass of white cabbage in LM systems (Båth et al., 2008);
 17 sowing LM after maize planting decreased the competition for nutrients and then increased the dry
 18 weight of the cash crop (Uchino et al., 2009); sowing LM 7 weeks or more after leek transplanting
 19 improved leek yield compared to 3 or 5 weeks (Kolota et al., 2004); and the use of the substitution
 20 design has given acceptable yields (Thorup-Kristensen et al., 2012).

21 The ability of LMs to reduce nitrate leaching has only been subject to few studies in row crop
 22 production compared to studies of weed and insect control and cash crop yield (Brainard and
 23 Bellinder, 2004). Moreover, studies have only been conducted in one site at a time, hindering
 24 general conclusions across climatic, edaphic and management conditions. To investigate the
 25 feasibility of LMs to reduce the risk of nitrate leaching in organic vegetables, cauliflower (*Brassica*

1 *olerace L.*) and leek (*Allium porrum L.*), two years of field trials with intercropping of LM and
 2 vegetables were conducted in Italy, Slovenia, Germany and Denmark, representing different
 3 cropping conditions across Europe. Introduction strategies of LM (sowing date and crop density) for
 4 competition control were investigated. Open-pollinated and hybrid cultivars of vegetables were
 5 used to reveal the competitiveness for N. We hypothesized that: 1) hybrid cultivars show higher
 6 ability to compete for N in the LM systems compared to open-pollinated cultivars; 2) due to higher
 7 competition for N, the addition design (ADD) is more effective in reducing soil N_{inorg} than the
 8 substitution design (SUB); 3) the delay of LM sowing late after cash crop planting, is more efficient
 9 in reducing the risk of nitrate leaching due to weaker competition for N in comparison to the sole
 10 crop and early-sown LM.

11 Materials and Methods

12 Experimental sites

13 This study was conducted in four European countries, Italy (2011 and 2012), Germany, Slovenia,
 14 and Denmark (2012 and 2013). The geographical locations of the four experimental sites were the
 15 research farm Hessian State Estate Frankenhäusen, Germany (51°9'N, 9°4'E), Agricultural Centre
 16 of the University of Maribor, Slovenia (46°28'N, 15°38'E), CREA - ORA research farm, in the
 17 Marche Region, Italy (42° 53' N, 13° 48' E) and the Research Centre Aarslev, Aarhus University,
 18 Denmark (55°18'N, 10°27'E). The soil properties of the four experimental sites are listed in Table 1.
 19 The annual precipitation and average air temperature during the vegetable growing season was 272
 20 mm and 13.4 °C and 634 mm and 13.5 °C and in Italy, 430 mm and 18.1 °C and 230 mm and
 21 18.4 °C in Slovenia, 293 mm and 11.9 °C and 257 mm and 13.5 °C in Germany, and 441 mm and
 22 14 °C and 167 mm and 16 °C in Denmark. The field experiments were conducted according to the
 23 local organic management practice and rules, in which inorganic fertilizers and synthetic pesticides
 24 were excluded.

1 The trials were conducted using randomized block design with three replicates in four countries,
 2 where cauliflower and leek (except Italy) were grown as cash crops. Different plant species
 3 (legumes or non-legumes) were chosen as LM. Open-pollinated (OP) cultivars and hybrids of the
 4 vegetables, the choice of LM and the means of controlling interspecific competition varied from
 5 country to country depending on local practice (Table 2). The management practices are listed in
 6 Table 3. The addition and substitution design were compared in Germany. The crop density in the
 7 addition design was the same as in the sole crop system (sole crop), and LM was sown in strips,
 8 which allowed weeding until LM sowing. In the substitution design every third row of vegetables
 9 was replaced by a row of LM, resulting in a reduction of crop density by 1/3 per area unit, which
 10 allowed weeding after LM sowing. To alleviate the competition between vegetables and LM, the
 11 displacement of sowing dates of LM was tested in Slovenia, Italy and Denmark. The LM in those
 12 trials was either early-sown or late-sown after vegetable planting. The LM used in the Danish
 13 cauliflower trial was an overwintering, grass-clover mixture, which was sown in the previous April
 14 and was either fully incorporated or incorporated in strips in the previous December according to
 15 Båth et al.(2008). Before cauliflower planting, the row of LM was cut aboveground and root pruned
 16 to 0.2 m soil depth on May 22, 2012 and May 14, 2013. After the vegetables were harvested, the
 17 LM was left in the field to grow until early spring in Italy, Slovenia, Germany and Denmark.

18 Plant and soil sampling and analysis

19 Cauliflower and leek were harvested by hand and were sorted into yield and crop residues. LM was
 20 sampled by cutting at the soil surface just before (Germany) or after (all others) the harvest of
 21 vegetables. All plant samples were oven-dried to a constant weight (Germany: 80 °C for 48h;
 22 Slovenia: 105 °C; Italy: 70 °C for 48 h; Denmark: 80°C for 20h) and were analyzed by the
 23 combustion method. Soil in the Slovenian, German and Danish trials was sampled 3 times per
 24 cropping cycle to determine the soil N_{inorg} representing the planting time, harvest and the end of the
 25 main leaching period the following spring. Soil samples were also taken at the start of the main

leaching period in autumn in the Danish trials and the German cauliflower trial in 2012 and leek trial in 2013. The start and end of the main leaching periods were defined according to local climate and soil conditions (Table 3). The soil samples were taken to a depth of 0.9 m (Slovenia and Germany), 1.5 m (Denmark, cauliflower) or 2.5 m (Denmark, leek) by manual or machine driven augers. Soil sampling and analysis for soil N_{inorg} (Germany NO_3^-) content was performed after standard agronomic procedures.

Data analysis

Soil N_{inorg} was calculated per area based on the measured soil N_{inorg} concentrations ($N-NO_3^-$ in Germany) and the local soil bulk densities for each soil layer. The results of the plant N content and the soil N_{inorg} distribution were analyzed by the Kenward-Roger based method (R software, version 3.0.2). The living mulch (LM), cultivars (Cultivar) and year (Year) were treated as fixed factors and blocks were the random effect. If the variances were not homogeneous, data were transformed by the function $y=X^{1/2}$ or $y=\log(x)$. Tukey's test was applied to compare the significance of the differences among treatments with $p<0.05$.

Results and Discussions

Cauliflower trials

The influence of LM, cultivar and year on the total crop and LM uptake of N in four countries is presented in Table 4. Interactions between LM, cultivar and/or year were found in the N uptake of cauliflower in Germany, Slovenia and Denmark. In Denmark, LM decreased cauliflower N considerably by 43 kg N ha^{-1} ($p<0.01$). In Italy, the hybrid (HF1) had higher crop N uptake and higher total N uptake (crop + LM) compared to two open-pollinated cultivars ($p<0.01$). The differences for cauliflower N amounted to 48 and 95 kg N ha^{-1} between the hybrid and open-pollinated cultivars. The early-sown LM system had 173 and 141 kg N ha^{-1} lower cauliflower N uptake compared to the sole crop and late-sown LM, respectively ($p<0.01$).

Figure 1 presents the results of the plant N uptake from the trials with cv. Chambord, which was tested in Germany, Slovenia and Denmark. The difference of total plant N uptake in each country was not consistent. Significant differences between treatments were only found in 2012 in Germany and Slovenia. The decrease of cauliflower N was observed in countries using the SUB design due to the reduced crop density, compared to the sole crop, but not in those using the ADD design. In the German and Danish trials, the SUB design had the lowest total cauliflower N uptake (cauliflower head + residues) compared to the other treatments (Figure 1), and this effect of reduced crop density was also observed in the reduced fresh biomass yields (results not shown). This result was in accordance with our hypothesis. No statistically significant differences were found in the cauliflower and residue N uptake between the ADD design and sole crop systems in Germany, Slovenia and Italy, when LM was sown late.

The SUB design was able to alleviate the interspecific competition compared to the ADD design. In 2013, the LM N uptake in the SUB design was higher than in the ADD design (Chambord: $14 \text{ kg N ha}^{-1} > 5 \text{ kg N ha}^{-1}$, $p < 0.05$). This was consistent with the other two cultivar treatments (White Ball: $34 \text{ kg N ha}^{-1} > 2 \text{ kg N ha}^{-1}$; Belot: $39 \text{ kg N ha}^{-1} > 0 \text{ kg N ha}^{-1}$, both $p < 0.05$) (results not shown). The LM N uptake in the ADD design was less than 5 kg N ha^{-1} in both years, indicating strong competition between the LM and cauliflower in this system due to the higher plant density. The SUB design was able to mitigate the interspecific competition and suppression on LM from cauliflower. The increase of LM N uptake compensated for the reduction of N uptake caused by the reduced cauliflower density. Similarly, the LM system of overwintering and root-pruned grass-clover mixture in Denmark had equivalent total N uptake to the sole crop.

The delay of LM sowing alleviated the suppression of cauliflower from the interspecific competition, compared to sowing LM at the same time as cauliflower planting. In Slovenia, where the addition design was used, the total cauliflower N of cv. Chambord in early-sown LM was 95 kg ha^{-1} lower than late-sown LM in 2012 and 113 kg ha^{-1} lower than the sole crop in 2013 (Figure 1).

1 Likewise, in cv. Snow Ball, early-sown LM had lower total cauliflower N than late-sown LM in
 2 2012 and sole crop in 2013 (Figure 2). In Italy, early sowing also decreased the total cauliflower N
 3 uptake in both years by 118-258 kg N ha⁻¹ with respect to the sole crop (Figure 3). This
 4 considerable suppression of N uptake in cauliflower grown with early-sown LM could be explained
 5 by the dependency of legumes on soil N_{inorg} at the early stage (Mahon et al., 1979). Therefore,
 6 sowing white clover (*Trifolium repens*) or black medic (*Medicago polymorpha* L.) at the same time as
 7 cauliflower transplanting led to strong competition between the crop and LM species, which was
 8 also confirmed by the lower fresh biomass yields (results not shown). In this case, the SUB design
 9 should be used to alleviate the competition to maintain the cauliflower yields. Another possibility is
 10 to use the ADD design combined with available management methods to control the interspecific
 11 competition during the crop season to maintain vegetable yield while reducing the risk of N_{inorg}
 12 leaching (see below). Such methods include root pruning (Båth et al., 2008) or mowing (Theriault et
 13 al., 2009), although the biomass partitioning of N indicated that mowing would not be efficient to
 14 control belowground competition. However, a system with LM root pruning in the ADD design
 15 poses technical challenges for the development of machinery due to the limited space. No
 16 difference was found in the cauliflower N between the sole crop and late-sown LM, which
 17 demonstrated that the delay of LM sowing, for instance, 4 weeks (Italy) or 2.5 weeks (Slovenia)
 18 after cauliflower transplanting strongly reduced interspecific competition compared to early-sown
 19 LM. Other studies also reported that the delay of LM or intercrop sowing can alleviate interspecific
 20 competition (Kolota and Adamczewska-Sowinska, 2004).

21 In general the LM did not accumulate as much N as expected based on a previous study, where
 22 white clover took up more than 63 kg N ha⁻¹ when grown as LM (Carof et al., 2007). The LM N
 23 uptake responded in a similar way to the delay of LM sowing (Table 4). No or only minor
 24 difference were found between early- and late-sown LM in Slovenia and Italy in all cultivars during
 25 both years (Figure 1, 2 and 3) due to the stronger competition in the early-sown LM system. In the

1 Italian trials, in the early-sown LM system, cauliflower N was 3.4 – 4.6 times lower than in the late-
 2 sown LM system, which led to lower total N uptake in the system with early-sown LM compared to
 3 late-sown LM in 2012. Different from our hypothesis on total N uptake, the delay of LM sowing in
 4 the cauliflower trials had higher total N uptake than early-sown LM system but was similar to the
 5 sole crop system. Thus, late-sown white clover or black medic increased the competitiveness of
 6 cauliflower, but resulted in no benefits for the total N uptake in the systems.

7 Cauliflower N uptake was higher in hybrid cultivars in Italy and Denmark (Table 4), but the
 8 performance of the cultivars could be related consistently to the sole crop and LM systems in any of
 9 the countries.

10 Soil N_{inorg} was measured in selected cultivars, systems and times in Germany, Slovenia and
 11 Denmark showing interactions of cultivar and year on total soil N_{inorg} at harvest in Germany (Tables
 12 5 and 8). In the Danish trials, the LM system had a lower total soil N_{inorg} of 33 kg N ha⁻¹ at the start
 13 of leaching compared to the sole crop ($p < 0.05$), indicating the potential of LM to reduce the risk of
 14 leaching during winter. The increase of soil N_{inorg} from the harvest to the start of leaching could be
 15 ascribed to the mineralization of soil organic N and cauliflower residues, which has been reported to
 16 increase soil N_{inorg} by 88 kg N ha⁻¹ after harvest (de Neve et al., 1998). The lower soil N_{inorg} in the
 17 LM system compared to the sole crop could be attributed to two factors. Firstly, the lower
 18 cauliflower density in LM resulted in lower cauliflower residue N. Secondly, LM left growing in
 19 the field after cauliflower harvest took up N and acted this way as an autumn catch crop.

20 Both the ADD and SUB designs in the German cauliflower trials reduced soil N_{inorg} at the start of
 21 leaching, and hypothesis 2) was rejected in most of cases in Germany when the two designs were
 22 compared. At the start of leaching, cv. Chambord had lower total soil N_{inorg} left in the ADD design
 23 (51 kg N ha⁻¹) compared to the sole crop and SUB design (68 and 65 kg N ha⁻¹) ($p < 0.05$) (Table 8).
 24 The two LM strategies with cv. Belot left less total soil N_{inorg} (12 and 20 kg N ha⁻¹ in ADD and SUB)

compared to the sole crop (45 kg N ha^{-1}) ($p < 0.01$) (Figure 6), which indicated that both LM systems had the potential to reduce soil N_{inorg} at the start of leaching by LM taking up N after harvest, considering no difference between treatments at the harvest. The difference was mainly present at the depth of 0.3-0.6 m for both cultivars and 0.6-0.9 m for Belot (Figure 6), which is within the expected root zone of white clover of 1.4 m (Evans, 1978). Thus, white clover took up N in the root zone after harvest.

In Slovenia in 2013, the sole crop system had lower total soil N_{inorg} than the early-sown LM (Table 8), which can be explained by the 113 kg N ha^{-1} less total N uptake in the early-sown LM ($p = 0.05$) (Figure 1); therefore, the importance of LM growth should be stressed to obtain a reduction in the risk of soil N_{inorg} leaching. Although not supported by the Slovenian results, the soil N_{inorg} in Denmark and Germany indicated the potential for a reduction of leaching risk by the introduction of LM in cauliflower systems.

Leek trials

Table 6 shows the effects of LM, cultivar and year on plant N accumulation. There were LM and year interactions for the total leek N uptake in Slovenia ($p < 0.01$) and the LM N uptake in all three countries. The total leek N uptake in Germany was affected by LM ($p < 0.01$) and the interaction between cultivar and year ($p < 0.05$). In the Danish trial, the leek N uptake was lower in both LM treatments compared to the sole crop treatment ($p < 0.01$). The total plant N uptake was influenced by the interaction between the LM and year in all three countries, which suggested that the effect of LM on total N uptake was influenced by the cropping conditions, which affected the growth of both leek and LM. An influence of leek cultivars on the total N uptake was only found in Denmark, and was higher in Hannibal compared to the hybrid Runner in Denmark ($p < 0.05$).

Figure 4 shows the results of plant N uptake in open-pollinated cv. Hannibal, which was tested in Slovenia, Germany and Denmark. Differences in total plant N uptake between treatments were found in Germany in 2012 and in Slovenia and Denmark in both years.

White clover as LM in Germany and Slovenia did not show consistent effects on total N uptake and the results varied from year to year, with positive effects of the SUB design in Germany 2013, which showed the potential of accumulating N by LM. The ADD design did not suppress leek N uptake compared to the sole crop system in Germany, but it did highly outcompete with both Hannibal and Striker in Slovenia in both years compared to the sole crop (Table 6, Figures 4 and 5). The results from Slovenia contradicted previous studies that reported that white clover was less competitive against leek compared to other LMs and that similar yield to the sole crop was achieved when white clover was sown after leek transplanting (Kolota and Adamczewska-Sowinska, 2004, den Hollander et al., 2007). For Hannibal in the SUB design in Germany, the leek N uptake was consistently lower than the sole crop in both years (2012: $p < 0.01$; 2013: $p < 0.05$) because of the reduced crop density (Figure 4). This was consistent with the leek N uptake in LM systems in the Danish trial with both cultivars.

The total N uptake of the LM system was comparable to the sole crop, except in Slovenia where the LM out competed the leek. In Germany in 2013, with an LM N of 27 kg ha^{-1} , the SUB design decreased the difference caused by leek N, and subsequently resulted in equivalent total N uptake (Figure 4). Similar results were observed with Axima in 2013 (data not shown). Overall, the ADD design achieved lower LM N uptake compared to the SUB design in 2013, with the three cultivars (data shown for Hannibal and Catcher, Figures 4 and 5) resulting similar total N uptake compared to the sole crop. When early-sown LM was established in Denmark 2013, it took up 48 and 44 kg N ha^{-1} with cv. Hannibal and Runner, respectively, and had comparable total N uptake to the sole crop (results shown for Hannibal in Figure 4).

1 The delay of white clover sowing might mitigate the suppression on leek compared to sowing LM
 2 at the same time as leek transplanting, where competition was strong, which was the case for the
 3 trials with the ADD design. In Slovenia, the leek N uptake of Hannibal and Striker in both years
 4 was extremely low and was even lower when LM was present compared to the sole crop in 2013,
 5 which suggested the suppression of LM on leek N uptake (Figures 4 and 5). The N uptake of LM in
 6 Slovenia was 5–13 fold that of the leek uptake, showing that the LM overgrew and suppressed the
 7 N uptake of leek to an extent that indicated failure of this experiment in Slovenia, which was
 8 confirmed by the results of the fresh biomass of leek (results not shown). This result was in contrast
 9 to the German trial in 2012, where leek suppressed the LM. The possible reason could be the
 10 different sowing dates of LM, which was one month after leek transplanting in Germany 2012 but
 11 only 2.5 weeks after leek transplanting in Slovenia, again stressing the importance of the
 12 displacement of sowing dates to the planting date of white clover when grown as an in-season LM
 13 during leek production. For example, Müller-Schärer (1996) demonstrated equivalent yields of leek
 14 when grown with ryegrass sown as LM at 6 weeks, but not 4 weeks, after leek transplanting. Kolota
 15 (2004) reported that the sowing of white clover should be delayed 7-11 weeks after leek
 16 transplanting for autumn harvested leek and 3-5 weeks for spring harvested leek. Due to the
 17 substitution design in Denmark, the N uptake of Hannibal was lower in both LM systems (Figure 4).
 18 For Runner in Denmark, 8 weeks, but not 5 weeks, delay of LM sowing allowed similar leek N
 19 uptake compared to the sole crop (not shown). Thus, the sowing date of white clover and dyers
 20 woad (*Isatis tinctoria*) had a large influence on the outcome of in-season intercropping with leek.
 21 The delay of sowing dyers woad decreased the LM N uptake compared to early-sown dyers woad
 22 and consequently had lower total N uptake in the Danish trials. This result contradicts with our
 23 hypothesis 3) of total N uptake. When LM grew well in 2013, the early-sown LM took up 48 kg N
 24 ha⁻¹ for Hannibal (Figure 4) and 44 kg N ha⁻¹ for Runner (results not shown), which resulted in
 25 equivalent total N uptake in the early-sown LM compared to the sole crop and higher N uptake than

late-sown LM ($p<0.05$). The delay of dyers woad sowing decreased the LM N uptake in both years and cultivars due to the short growing period, and resulted in lower total N uptake compared to the sole crop in 2013. This result verified the conclusion of the importance of sowing dates for N accumulation found by others (Vos et al., 1997). Overall, well-developed LM in the substitution design can compensate the N uptake reduction caused by lower leek density and achieve comparable total N uptake to the sole crop.

Soil N_{inorg} was measured in Hannibal at harvest and the end of leaching in the three countries and at the start of leaching in Denmark and Germany in 2013 (Table 7 and 8, $N-NO_3^-$ for Germany), showing interactions of cultivar, LM and/or year on total soil N_{inorg} at harvest in Germany (Table 7). The results from separate years showed that differences between systems were observed in the total soil N_{inorg} in Slovenia 2012 and Denmark in 2013 and in $N-NO_3^-$ in Germany 2012 (Table 8).

In the Danish trials in 2013, lower soil N_{inorg} was found at harvest in the early-sown LM compared to the two other systems in the soil layer of 0.3 – 1 m (early-sown LM: 68 kg N ha⁻¹; late-sown LM and sole crop: 109 and 102 kg N ha⁻¹; $p<0.05$) (Figure 7). At the start of leaching, early-sown LM left 145 kg N_{inorg} ha⁻¹ in the 0-2.5 m soil layer, which was lower than the 191 kg N ha⁻¹ in the late-sown LM calculated for both years (Table 7). These results are not in agreement with hypothesis 3) on reducing the risk of nitrate leaching. The soil N_{inorg} distribution with depth showed a significant difference at the 0.7-1 m soil layer at the start of leaching (Figure 6). This soil depth was out of the reach of the leek roots, which extend to a maximum of 0.5 m (Kristensen et al., 2007). Therefore, dyers woad, with root distribution to 2 m, took up soil N_{inorg} and decreased soil N_{inorg} in this soil layer (Xie et al., under review). These differences almost disappeared by the following spring at the end of the leaching season, when only early-sown LM had lower soil N_{inorg} than other systems in the soil layer of 1-1.5 m (not shown).

In Germany, neither the SUB nor the ADD design showed reduction of soil N at harvest each year, which was not in accordance with hypothesis 2). In Denmark, the delay of sowing dyers woad decreased the ability of cropping systems to deplete soil N. These results could be ascribed to the lower total N uptake, for example with the lowest total N uptake and the highest soil N_{inorg} in the SUB design in Germany 2012; and the lowest total N uptake in the late-sown LM system in Denmark in 2013 with the highest soil N_{inorg} content. Similar results were also found in some cases of cauliflower trials.

Conclusions

It was possible to use different introduction strategy of LM to control plant competition and to reduce the risk of N_{inorg} leaching, but the performance depended on the intensity of the interspecific competition and the local conditions. Some combinations of local conditions, cultivars and management options reduced the risk of N_{inorg} leaching. In most cases, the effect of legumes (white clover, burr medic) on reducing soil N_{inorg} was limited due to the poor growth or overgrowth of the legumes sown at the same time as cauliflower or leek transplanting, which had a negative effect on cash crop N uptake. Late sowing of legumes increased the competitiveness of the cash crop, whereas N accumulation of LM (legumes, dyers woad) decreased or was equal to the early-sown LM. In cauliflower trials, a month delay of sowing white clover decreased the soil N_{inorg} at the start of leaching by 17-33 kg ha⁻¹ in the ADD design and by 25 kg ha⁻¹ in the SUB design in Germany, and an overwintering and root-pruned mixture of grass-clover reduced soil N_{inorg} at the start of leaching by 33 kg N ha⁻¹ in Denmark. Dyers woad showed potential as LM by decreasing soil N_{inorg} by 52 and 38 kg N ha⁻¹ at harvest and at the start of leaching in leek production, when it was sown 5 weeks after leek transplanting. Leaving LM in the field until the following spring was a feasible option to reduce soil N_{inorg} before winter and to reduce the leaching risk in continental and temperate areas. The SUB design was better than the ADD design when the interspecific

competition intensity was strong. Sowing LM at the same time as the crop in the SUB design or in new ADD systems with root pruning to control the competition should be tested.

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Table 1. The soil properties of the experimental fields in four countries.

		C (%)	Clay (%)	Silt (%)	Sand (%)	P (mg kg ⁻¹)	K (mg kg ⁻¹)	pH _{CaCl2}
Italy								
0-0.25 m		-	20	28	52	27	312	8.1
0.25-0.7 m		-	21	25	54	6	175	8.4
1-1.1 m		-	15	19	66	-	-	-
Slovenia								
0-0.3 m		1.5	-	-	-	20.7	17.3	6.1
Germany								
0-0.3 m	Cauliflower	-	-	-	-	63.3	69.5	6.6
	Leek					52.6	61.3	6.6
0.3-0.6 m	Cauliflower	-	-	-	-	32.5	49.5	6.7
	Leek					18.8	48.0	6.7
Denmark								
0-0.5 m		1.0	13	15	70	23	115	6.7
0.5-1 m		0.2	18	13	68	19	98	5.9
1-1.25 m		0.1	18	14	68	16	105	7.3

The symbol - means the data are not available or determined.

Table 2. The cultivars of vegetables, the LM species and introduction strategy, and management of interspecific competition in four countries.

			Italy	Slovenia	Germany	Denmark
Cauliflower trial	Cultivar	Open pollinated	ORA1, ORA2	Snow Ball	White Ball	Goodman
		Hybrid	Emeraude HF1	Chambord	Chambord, Belot	Chambord
	LM species (seeding rate)		Burr medic (<i>Medicago polymorpha</i> L. var <i>anglona</i>) (80 kg ha ⁻¹)	White clover (<i>Trifolium repens</i> L.) (18 kg ha ⁻¹)	White clover (8 kg ha ⁻¹)	2012: Italian ryegrass (<i>Lolium multiflorum</i>) (5 kg ha ⁻¹), white clover (3 kg ha ⁻¹), black medick (<i>Medicago lupulina</i>) (3 kg ha ⁻¹) 2013: Italian ryegrass (5 kg ha ⁻¹), red clover (<i>Trifolium pratense</i>) (3 kg ha ⁻¹) black medick (3 kg ha ⁻¹)
	LM design		Addition	Addition	Addition and substitution	Substitution
	Management of competition		LM sowing date	LM sowing date	LM sowing date	Root pruning (not tested)
Leek trial	Cultivar	Open pollinated		Hannibal	Blaugrüner Herbst Axima, Hannibal	Hannibal
		Hybrid		Striker	Catcher F1	Runner
	LM species (seeding rate)			White clover (18 kg ha ⁻¹)	White clover (8 kg ha ⁻¹)	Dyers woad (100 seeds m ⁻¹)
	LM design			Addition	Addition and substitution	Substitution
	Management of competition			LM sowing date	LM sowing date	LM sowing date

Table 3. Agricultural management of crops and living mulches in four countries. If years are not indicated, the time point was the same in both years.

		Italy	Slovenia	Germany	Denmark
Cauliflower trial	Plot size	2.4 m×7 m	3.6 m×3.2 m	SUB: 4.5 m×18 m ADD: 4.5m × 10 m (2012) SUB: 3 m×18 m ADD: 3 m×10 m (2013)	3.2 m×6.5 m
	Row distance	0.7 m	0.6 m	0.75 m	0.53 m
	Plant distance	0.6 m	0.4 m	0.45 m	0.5 m
	N fertilizer (kg ha ⁻¹)	130	180 (organic mineral fertilizer from plant origin)	200 (spring inorganic N and manure N)	230 (spring inorganic N and manure N) (chicken manure)
	Cauliflower transplanting	Mid August	June 22 (2012) June 20 (2013)	June 28 (2012) June 27 (2013)	May 22 (2012); May 14 (2013)
	LM sowing and incorporation	Early sowing	Mid August	June 22 (2012) June 20 (2013)	April 1
		Late sowing	Early September (2011) Mid September (2012)	July 10	July 26 (2012) August 7 (2013)
		Incorporation			Previous December
	Cauliflower harvest	Mid December to late February, 2012 (2011) Mid November to late December (2012)	Late October	Early September (CH); Mid September (WB); Mid November (BE)	Early August to late August (2012); Late July to early August (2013)
	LM sampling	Harvest	Late October	Late August (CH); Mid September (WB); Early November (BE)	Late August (2012); Mid August (2013)
		Start of leaching	Late September (2011) Mid November (2012)		Mid November (2012); Early November (2013)
		Harvest	*	October (0-0.9 m)	August 23 (2012) August 20 (2013) (0-1.5 m)
	Soil sampling	Start of leaching		October (0-0.9 m)	November 13 (2012); November 5 (2013) (0-1.5 m)
				Mid November (2012, CH, WB); Mid December (2012, BE) (0-0.9 m)	

		End of leaching	April (0-0.9 m)	Early March (2012); Late March (2013) (0-0.9 m)	April 2 (2013); March 19 (2014) (0-1.5 m)
Leek trial	Plot size		3.6 m×3.2 m	ADD: 4.5 m×4.0 m SUB: 4.5 m×6.0 m (2012) ADD: 3.0 m×4.0 m SUB: 3.0×6.0 m (2013)	3.2 m×6.5 m
	Plant distance		0.15 m	0.11 m	0.08 m
	N fertilizer (kg ha ⁻¹)		126 (organic mineral fertilizer from plant origin)	200 (spring inorganic N and applied N (residues of maize starch extraction)	200 (spring inorganic N and manure N) (chicken manure)
	Leek transplanting		Late June	June 28 (2012) June 27 (2013)	May 25 (2012) May 31 (2013)
	LM sowing	Early	Late June	July 26 (2012) August 7 (2013)	July 4 (2012) July 5 (2013)
		Late	Early July		July 23 (2012) July 26 (2013)
	Leek harvest	Harvest	Late October	2012: Late October (HA); Early November (CA); Mid November (AX) 2013: Late September (HA); Mid October (CA, AX) As same as leek harvest	Early October(2012) Late September (2013)
	LM sampling	Harvest Start of leaching			As same as leek harvest Late November
	Soil sampling	Harvest Start of leaching	October (0-0.9 m)	Early November (2012) Late October (2013) (0-0.6 m) Early October (2013, HA) Mid October (2013, CA, AX) (0-0.9 m)	Late October (2012) Early October (2013) (0-2.5 m) Late November (0-2.5 m)
		End of leaching	April (0-0.9 m)	Early March (2012) Late March (2013) (0-0.9 m)	Early April (2013) Late March (2014) (0-2.5 m)

Note: *Soil N_{inorg} was studied by use of lysimeters in Italy, but inadequate amounts of soil solution disabled statistical analysis. SUB: Substitution design, ADD: Addition design. The abbreviations in parentheses indicate the cultivar of cauliflower or leek (CH: cauliflower cv. Chambord; WB: cauliflower cv. White Ball; BE: cauliflower cv. Belot; HB: leek cv. Hannibal; AX: leek cv. Axima; CA: leek cv. Catcher).

Table 4. Effect of living mulch, year and cultivar on cauliflower N uptake in the four Countries.

	Italy		Slovenia		Germany		Denmark	
	Cauliflower N	LM N	Cauliflower N	LM N	Cauliflower N	LM N	Cauliflower N	LM N
Living mulch								
No LM	233.2 A		259.8		223.2		132.5 A	
LM 1	60.0 (es) B	24.4 (es)	136.4 (es)	32.6 (es)	219.0 (ADD)	4.8 (ADD)	89.3 B	31.6
LM 2	200.9 (ls) A	14.1 (ls)	218.2 (ls)	31.3 (ls)	184.4 (SUB)	22.5 (SUB)		
Year								
2012	171.7	14.3	186.6	14.3 b	209.1	9.4	113.4	28.7
2013	157.7	24.2	227.7	41.1 a	208.6	18.8	108.3	34.5
Cultivar								
Open Pollinated 1	164.6 (Ora1) b	17.5 (Ora1)	200.7 (SB)	34.4 (SB)	221.3 (WB)	14.9 (WB)	98.9 (GM)	32.2 (GM)
Open Pollinated 2	117.3 (Ora2) B	27.0 (Ora2)	-	-	-	-	-	-
Hybrid	212.3 (HF1) Aa	13.3 (HF1)	208.9 (CH)	30.0 (CH)	160.3 (CH)	8.3	122.9 (CH)	31.0 (CH)
					245.0 (BL)			
LM	***			n.s.		n.s.	***	n.s.
Cultivar	***	n.s.	n.s.	n.s.		n.s.		n.s.
Year	n.s.			*		n.s.		n.s.
LM x Year	n.s.	*	**	n.s.		n.s.	n.s.	n.s.
Cultivar x Year	n.s.	n.s.	n.s.	n.s.		n.s.	*	n.s.
LM x Cultivar x Year	n.s.	n.s.	n.s.	n.s.	***	n.s.	n.s.	n.s.

Note: The * indicates the significant difference at 0.05 level, ***indicates the significant difference at 0.001 level. Different lower case letters following the numbers indicate the significant difference ($p < 0.05$) and the upper case letters indicate the significant difference ($p < 0.01$). The abbreviations given in parentheses indicate the design (ADD: Addition design; SUB: Substitution design); sowing date of living mulch (es: Early sown; ls: Late sown) or cauliflower cultivar (WB: White Ball; CH: Chambord; BE: Belot; HF1: Emeraude HF1; SB: Snow Ball; GM: Goodman).

Table 5. The effect of LM, cultivar and year on the total soil inorganic N (kg ha⁻¹) in cauliflower trials at harvest, start of leaching and end of leaching.

	Slovenia		Germany		Denmark		
	Harvest	End of leaching	Harvest	End of leaching	Harvest	Start of leaching	End of leaching
Living mulch							
No LM	69.6	388.1	49.8	44.3	79.5	151.6 a	130.9
LM 1	72.0 (es)	199.0 (es)	40.7 (ADD)	42.6 (ADD)			
LM 2			44.9 (SUB)	48.3 (SUB)	82.3 (SUB)	118.1 (SUB) b	110.2 (SUB)
Cultivar							
Open pollinated	-	-	60.7 (WB)	45.6(WB)	-	-	-
Hybrid 1	-	-	52.9 (CH)	48.1 (CH)	-	-	-
Hybrid 2	-	-	21.9 (BE)	41.5 (BE)	-	-	-
Year							
2012	81.5	528.7	56.6	44.5	94.0	118.2	122.8
2013	60.1	58.5	33.8	45.6	67.8	151.5	118.2
LM	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.
Cultivar x Year	n.s.	n.s.	*	n.s.	-	-	-
LM x Cultivar	n.s.	n.s.	n.s.	n.s.	-	-	-

Note: The * indicates the significant difference at the level of 0.05. Different lower case letters following the numbers indicate the significant difference ($p < 0.05$). The abbreviations given in parentheses indicate the design (ADD: Addition design; SUB: Substitution design); sowing date of living mulch (es: Early sown; ls: Late sown) or cauliflower cultivar (WB: White Ball; CH: Chambord; BE: Belot). The soil inorganic N was measured in the soil layer of 0-0.9 m in Germany (except 2012 at harvest 0-0.6 m), 0-0.9 m in Slovenia and 0-1.5 m in Denmark. In Slovenia and Denmark, the soil N_{inorg} results were measured for Chambord.

Table 6. The leek (including residues) and living mulch (LM) N uptake (kg N ha⁻¹) in three countries in two years.

	Slovenia		Germany		Denmark	
	Leek N	LM N	Leek N	LM N	Leek N	LM N
Living mulch						
No LM	26.0		176.2 A		103.0 A	
LM1	3.5 (es)	50.4 (es)	164.8 A (ADD)	5.6 (ADD)	68.1 (es) B	30.5 (es)
LM2	6.2 (ls)	101.2 (ls)	120.4 B (SUB)	17.7 (SUB)	77.1 (ls) B	8.6 (ls)
Year						
2012	8.3	25.8	176.0	1.1	85.9	10.9
2013	15.5	88.0	131.6	21.4	79.6	28.3
Cultivar						
Open Pollinated	9.8 (HB)	67.1 (HB)	148.6 (HB)	9.2 (HB)	86.3 (HB) a	19.4 (HB)
Hybrid 1	14.0 (ST)	67.5 (ST)	149.9 (AX)	10.1 (AX)	79.2 (RN) b	19.8 (RN)
Hybrid 2			163.0 (CT)	11.5 (CT)		
LM			***		***	
Cultivar	n.s.	n.s.	n.s.	n.s.	*	n.s.
Cultivar x Year	n.s.	n.s.	*	n.s.	n.s.	n.s.
LM x Year	***	**	n.s.	***	n.s.	***

Note: The *, ** and *** indicate the significant difference at 0.05, 0.01 and 0.001 level, respectively. Different lower case letters following the numbers indicate the significant difference ($p < 0.05$) and the upper case letters indicate the significant difference ($p < 0.01$). The abbreviations given in parentheses indicate the design (ADD: Addition design; SUB: Substitution design); sowing date of living mulch (es: Early sown; ls: Late sown) or leek cultivar (HB: Hannibal; AX: Axima; CT: Catcher; ST: Striker HF1; RN: Runner).

Table 7. The effect of LM, cultivar and year on the total soil inorganic N (kg ha⁻¹) in leek trials at the harvest, the start of leaching and the end of leaching.

	Slovenia		Germany		Denmark		
	Harvest	End of leaching	Harvest	End of leaching	Harvest	Start of leaching	End of leaching
Living mulch							
No LM	91.7	45.2	41.6	61.1	214.5	171.0 AB	118.2
LM 1	57.9 (es)	45.8 (es)	43.8 (ADD)	60.7 (ADD)	156.8 (es)	144.6 B(es)	103.4 (es)
LM 2			57.5 (SUB)	64.28 (SUB)	190.6 (ls)	190.7 A(ls)	114.3 (ls)
Cultivar							
Open pollinated			55.8 (HB)	63.0 (HB)			
Hybrid 1			39.7 (AX)	63.2 (AX)			
Hybrid 2			47.4 (CT)	60.1 (CT)			
Year							
2012	86.7	46.0	43.5	55.2	160.9	145.6 b	105.9
2013	62.9	45.0	51.8	71.1	213.7	192.0 a	118.1
LM	n.s.	n.s.			n.s.	**	
Year	n.s.	n.s.			n.s.	*	
LM x Cultivar	-	-		*	-	-	-
Cultivar x Year	-	-		*	-	-	-
LM x Year	n.s.	n.s.		n.s.	n.s.	n.s.	n.s.
LM x Year x Cultivar	-	-	*	n.s.	-	-	-

Note: The * indicates the significant difference at 0.05 level. The abbreviations given in parentheses indicate design (ADD: Addition design; SUB: Substitution design); sowing date of living mulch (es: Early sown; ls: Late sown) or leek cultivar (HB: Hannibal; AX: Axima; CT: Catcher). The soil inorganic N was measured in the soil layer of 0-0.9 m in Germany (except 2012 at harvest 0-0.6 m), 0-0.9 m in Slovenia and 0-2.5 m in Denmark. The soil N_{inorg} results from Slovenia and Denmark was only for Hannibal.

Table 8. The effect of LM on soil inorganic N (kg ha⁻¹) in trials with cauliflower cultivar Chambord and leek cultivar Hannibal at harvest, at the start of leaching and at the end of leaching in separate years.

		Treatment	Harvest		Start of leaching		End of leaching	
			2012	2013	2012	2013	2012	2013
Cauliflower	Slovenia	No LM	90.8	48.3 B			723.2	53.1
		Es LM	72.1	71.9 A			334.2	63.8
	Germany	No LM	51	41.7	68.0 a		48.3	46.3
		ADD	44.7	50	51.3 b		40.3	46.3
		SUB	34.3	42.3	65.3 a		54.7	52.3
	Denmark	No LM	94.3	64.7	125.3	177.8	126.4	135.3
		LM	93.8	70.9	111.0	125.2	119.3	101.1
Leek	Slovenia	No LM	119.2a	64.2			53.9	36.5
		Es LM	54.1 b	61.6			38.0	35.7
	Germany	No LM	31.0 b	50.7		51.7	49.3	71.3
		ADD	32.0 b	48.0		73.3	46.3	56.0
		SUB	121.0 a	52.3		74	70.7	84.3
	Denmark	No LM	198.2	211.8 ab	143.4	179.9 ab	107.3	115.1
		Es LM	139.1	160.1 b	131.0	141.7 a	94.3	100.6
		Ls LM	131.2	233.2 a	148.9	209.7 b	95.2	120.2

Note: Different lower case letters following the numbers indicate the significant difference ($p < 0.05$). Different upper case letters following the numbers indicate the significant difference ($p < 0.01$). The abbreviations given in the table indicate design (No LM: sole crop; ADD: Addition design; SUB: Substitution design) or sowing date of living mulch (es: Early sown; ls: Late sown).

Figure 1. The plant N uptake in cauliflower trials with hybrid cultivar Chambord in Germany, Slovenia and Denmark. Different lower case letters indicate the significant difference ($p < 0.05$). Different upper case letters indicate the significant difference ($p < 0.01$). No LM: Sole crop; ADD: Addition design; SUB: Substitution design; Es LM: Early sown LM; Ls LM: Late sown LM.

Figure 2. The plant N uptake in cauliflower trials in Slovenia with cv. Snow Ball in two years. Different lower case letters indicate the significant difference ($p < 0.05$). Different upper case letters indicate the significant difference ($p < 0.01$). No LM: Sole crop; Es LM: Early sown LM; Ls LM: Late sown LM.

Figure 3. The plant N uptake in cauliflower trials in Italy with cv. Hf1e, ora1 and ora2 in both years. Different lower case letters indicate the significant difference ($p < 0.05$). Different upper case letters indicate the significant difference ($p < 0.01$). No LM: Sole crop; Es LM: Early sown LM; Ls LM: Late sown LM.

Figure 4. The plant N uptake in leek trials with open pollinated cv. Hannibal in Germany, Slovenia and Denmark. Different lower case letters indicate the significant difference ($p < 0.05$). Different upper case letters indicate the significant difference ($p < 0.01$). No LM: Sole crop; ADD: Addition design; SUB: Substitution design; Es LM: Early sown LM; Ls LM: Late sown LM.

Figure 5. The plant N uptake in leek trials with hybrid cv. Catcher from Germany and Striker F1 from Slovenia. Different lower case letters indicate the significant difference ($p < 0.05$). Different upper case letters indicate the significant difference ($p < 0.01$). No LM: Sole crop; ADD: Addition design; SUB: Substitution design; Es LM: Early sown LM; Ls LM: Late sown LM.

Figure 6. The soil N_{inorg} distribution in the soil layer of 0-0.9 m in cauliflower trials in Germany 2012 at the start of leaching. The * indicates the significant difference at 0.05 level. No LM: -Sole crop; ADD: Addition design; SUB: Substitution design.

Figure 7. The soil N_{inorg} distribution in the soil layer of 0-1 m at harvest and at the start of leaching in leek trials in Denmark 2013. The * indicates the significant difference at 0.05 level. No LM: Sole crop; Es LM: Early sown LM; Ls LM: Late sown LM.