Europ. J. Agronomy 82 (2017) 21–32

Contents lists available at ScienceDirect

European Journal of Agronomy

journal homepage: www.elsevier.com/locate/eja

Intercropping leek (*Allium porrum* L.) with dyer's wood (*Isatis tinctoria* L.) increases rooted zone and agro-ecosystem retention of nitrogen

Yue Xie, Hanne Lakkenborg Kristensen*

Department of Food Science, Aarhus University, 5792 Aarslev, Denmark

A R T I C L E I N F O

Article history:
Received 12 January 2016
Received in revised form
20 September 2016
Accepted 26 September 2016
Available online 15 October 2016

Keywords:
Intercropping
Organic leek
Soil N_{inorg}
Root distribution
Competition

A B S T R A C T

Nitrate leaching can be high in organic vegetable production. Late-harvested crops like leek limit the use of autumn catch crops. The aim of this study was to investigate the growing of a combination of a deep-rooted catch crop and a shallow-rooted vegetable to reduce the risk of nitrate leaching. We compared a leek sole crop (S) with two intercropped systems of leek and early-sown dyer's wood (five weeks after leek planting) (IE) or late-sown dyer's wood (eight weeks after leek planting) (IL) in two seasons: 2012 and 2013. To reveal root and resource competition, leek with dyer's wood rows left empty (S_{emp}), and early and late-sown dyer's wood with leek rows left empty (DE_{emp}, DL_{emp}) were included. Yield, dry aboveground biomass, aboveground N accumulation and soil inorganic N (N_{inorg}) were measured as well as root growth by use of minirhizotrons to 2.3 m soil depth. Results showed that the marketable yield of leek in IE and IL systems was comparable with the yield in the S system when calculated per length of leek row. The Relative Competition Index (RCI) revealed that interspecific competition facilitated the growth of leek but hampered that of dyer's wood. The rooted zone increased from 0.5 m in the S system to more than 2 m depth in those of the intercropped systems. Dyer's wood ceased growing above ground but kept growing below ground after crop harvest and extended roots under the leek root system in 2012. Intercropping increased the root intensity of late-sown dyer's wood after leek harvest in the 0.75–1.75 m soil layer compared to dyer's wood growing alone (DL_{emp}), while the root depth was not affected. The intercropped system with early-sown dyer's wood reduced soil N_{inorg} by 52 kg ha^{-1} relative to the sole-cropped system, and dyer's wood accumulated 48 kg N ha^{-1} in aboveground biomass at harvest in 2013. Late-sown dyer's wood had fewer roots, left higher soil N_{inorg} and had lower aboveground N accumulation than early-sown dyer's wood until the following spring. Therefore, early-sown dyer's wood is applicable in an organic intercropped system with high yields of leek to decrease the risk of nitrate leaching.

1. Introduction

Organic vegetable production relies on mineralization of organic N sources, but may still have high nitrate leaching to the environment. This is due to the mismatch of the timing in mineralization of N and plant N uptake between seasons, and the growing of vegetables with high N demand but low N use efficiency. Catch crops grown after crop harvest are known for reducing nitrate leaching and improving N use efficiency in field crop rotations as well as in vegetable production (Tuulos et al., 2015; Wyland et al., 1996). However, late-harvested crops, such as leek (*Allium porrum* L.), may leave insufficient time for catch crop growth and N uptake before winter, which increases the risk of nitrate leaching (Kristensen and Thorup-Kristensen, 2007).

Intercropped systems with a cash crop and a catch crop growing in alternating rows allow the catch crop to take up N during the crop growth season and after crop harvest. This has been shown to reduce the risk of nitrate leaching in organic cropping system (Thorup-Kristensen et al., 2012), but reports of the effect of intercropped catch crops on N cycling are scarce. Autumn catch crops with high N-sink capacity have been reported to take up more N from the soil and prevent nitrate leaching, compared to fallow. For example, winter rape (*Brassica napus* L.) and fodder radish (*Raphanus sativus* L. var. *oleiformis* Pers.) were found to take up 127 and 167 kg N ha^{-1} from August to November (Thorup-Kristensen, 1994). Additionally, the root depth and subsoil root intensity of catch crops were found to have a strong correlation with soil N_{inorg} left for leaching in the subsoil (Thorup-Kristensen and Rasmussen, 2015). Species from the *Brassica* family are well known for their

---

* Corresponding author.
E-mail address: hanne.kristensen@food.au.dk (H.L. Kristensen).

http://dx.doi.org/10.1016/j.eja.2016.09.017

deep root systems, which play an important role in depleting N_{inorg} in deep soil layers. For example, fodder radish with root depth of more than 2.4 m could take up N from more than 2 m depth (Kristensen and Thorup-Kristensen, 2004). Apart from the genetic traits, the root growth is influenced by the growing environment such as the availability and distribution of N (Kristensen and Thorup-Kristensen, 2007), phosphorus (Kang et al., 2014) and water (Ebrahimi et al., 2014) as well as the presence of neighboring plants (Hauggaard-Nielsen et al., 2001). However, studies on the effect of deep-rooted catch crops on root growth in intercropped systems are scarce.

Attention should also be paid to the interspecific competition in intercropped systems for nutrients, light and water, which may hamper crop growth. Strategies for catch crop management, like appropriate choice of species, mowing (Theriault et al., 2009), pruning the roots (Båth et al., 2008) and delayed sowing (Vanek et al., 2005) as well as maintaining overall plant density (Thorup-Kristensen et al., 2012) could reduce the interspecific competition and result in acceptable yields.

Dyer's wood (*Isatis tinctoria* L.) was cultivated throughout Europe as a source of blue dye, but is considered a noxious weed in the western United States (Dewey et al., 1991). Its low plasticity in response to changes in soil N indicated that it has low N requirements or low N productivity (Monaco et al., 2005), suggesting that it may be less competitive for N compared to many cash crops. Due to its root depth of 2.4 m, it reduced the subsoil nitrate to 15 kg N ha^{-1}, compared to the 62 kg N ha^{-1} without a catch crop (Thorup-Kristensen and Rasmussen, 2015). On the contrary, leek is known to have a shallow root system with a root depth of 0.5 m leaving a higher amount of soil N_{inorg} for leaching in the autumn compared to deep-rooted vegetables (Kristensen and Thorup-Kristensen, 2007). We hypothesized that combining deep-rooted dyer's wood with shallow-rooted leek would reduce the risk of nitrate leaching. Since delayed sowing of catch crops relative to cash crops may increase the competitiveness of cash crops by allowing them to dominate for nutrients and space (Vanek et al., 2005), we hypothesized that delayed sowing of dyer's wood would also be an effective tool to increase the competiveness of leek.

Therefore, we aimed to investigate the feasibility of introducing dyer's wood as intercrop to reduce the risk of nitrate leaching, and the effect of delayed sowing of dyer's wood to control the interspecific competition. The hypotheses in the present study are: 1) Early sown dyer's wood has strong competiveness against leek and affects leek yield, while late sown dyer's wood reduces the interspecific competition having less roots and biomass, compared to early sown dyer's wood. 2) Introducing dyer's wood reduces the risk of nitrate leaching after harvest, while the effect is reduced by delayed sowing of dyer's wood. 3) Dyer's wood increases the soil volume explored by roots compared to the sole-cropped system, and grows beneath the leek root system. 4) Dyer's wood keeps growing and taking up soil N_{inorg} after leek harvest. 5) Dyer's wood develops a deeper root system in the intercropped system than when grown alone.

We tested these hypotheses in a two-year field experiment in which we assessed leek crop yields and biomass of all plants, root distribution, plant N accumulation and soil N_{inorg} in treatments with and without a dyer's woad catch crop, and in which sowing of the catch crop occurred at five and eight weeks after leek planting.

2. Material and methods

2.1. Field sites and experimental design

A field experiment was conducted during two cropping cycles in 2012 and 2013 at the Research Centre Aarslev, Denmark (10°27′E, 55°18′N) on a sandy loam (Typic Agrudalf) which contained 9 g C kg^{-1}, 134 g kg^{-1} clay, 151 g kg^{-1} silt and 696 g kg^{-1} sand at the 0–0.5 m soil layer; 2 g C kg^{-1}, 188 g kg^{-1} clay, 132 g kg^{-1} silt, and 676 g kg^{-1} sand at the 0.5–1 m soil layer; 2 g C kg^{-1}, 181 g kg^{-1} clay, 138 g kg^{-1} silt, and 678 g kg^{-1} sand at the 1–2.5 m soil layer. The P content was 24, 19 and 16 mg kg^{-1} and the K content was 119, 102 and 105 mg kg^{-1} at the soil layers of 0–0.5, 0.5–1 and 1–2.5 m. The pH_{CaCl2} value was 6.8, 5.9 and 7.3. The mean annual air temperature and mean precipitation were 8.5 °C and 664 mm recorded at the meteorological station at the research center, Aarslev (1987–2012). Daily mean temperatures and precipitation during the experimental period are shown in Fig. 1. The field management was according to the Danish organic management regulations since 1996 without use of pesticides or inorganic fertilizers.

The experiment was run in 2012 and 2013 at two adjacent fields with a distance of 286 m. In the 2012 experiment a mixture of perennial ryegrass (*Lolium perenne* L.), red clover (*Trifolium pratense* L.) and black medick (*Medicago lupulina* L.) had been sown in late April, 2010 and incorporated in late July, 2011. Then fodder radish was sown as an autumn catch crop in early August, 2011 and incorporated into the soil in early April, 2012. In the 2013 experiment, the same mixture of grass and legumes had been sown in late April, 2011 and incorporated in December, 2012.

Each year a completely randomized block design with three replicates was applied. The plot size was 3.2 m × 6.5 m. Six rows of plants were planted in one plot with a row distance of 0.53 m and plant distance of 0.08 m. Leek (open pollinated cv. Hannibal) was germinated and grown under organic greenhouse conditions since March 9, 2012 and March 13, 2013. The leeks were transplanted to the field on May 25, 2012 and May 31, 2013 and grown either as a sole crop (S), an intercrop with every third row replaced by a row of dyer's woad (I) or as a sole crop with every third row left empty (S_{emp}). Although not relevant for growers the S_{emp} system was included to be able to separate effects of changes in leek density between the systems. The dyer's woad was sown at 100 germinating seeds per meter row at two different dates denoted as early sowing (IE) (July 4, 2012; July 5, 2013), and late sowing (IL) (July 23, 2012; July 26, 2013). The design with a row of dyer's woad replacing a row of leek is called a substitutive design. In order to observe the effect of intercropping on the root growth of dyer's woad, an additional system was included where dyer's woad was grown solely in every third row at early sowing dates (DE_{emp}) and late sowing dates (DL_{emp}) with empty crop rows. The amount of N fertilizer in the form of dried chicken manure applied was 70 and 123 kg ha^{-1} in 2012 and 2013 respectively, reaching to a total of 200 and 210 kg N ha^{-1} (soil N_{inorg} and N fertilizer) right after leek transplanting. The density of leek was 23 plants m^{-2} in the S system and 15.6 plants m^{-2} in the IE, IL and S_{emp} systems due to the lower number of rows. Over the growing season the leeks were irrigated at moderate soil water deficits. The total amount of water given was equivalent to 65 and 150 mm of precipitation in 2012 and 2013, respectively.

2.2. Root measurements

The root growth was registered over time by use of the minirhizotron method, which were transparent plastic tubes of 3 m length. In each plot, two minirhizotrons were inserted into the soil at an angle of 36° from vertical to reach 2.3 m depth per plot. Details of the method are given in (Kristensen and Thorup-Kristensen, 2004). The minirhizotrons were installed in the leek, dyer's woad and empty rows within a few days after planting/sowing in the systems to reveal the effect of intercropping on root growth. Two counting grids (40 mm × 40 mm crosses) had been drawn on the upper side of each minirhizotron. The roots in the grids were recorded by a mini-video camera. Root density was registered as intensity of the

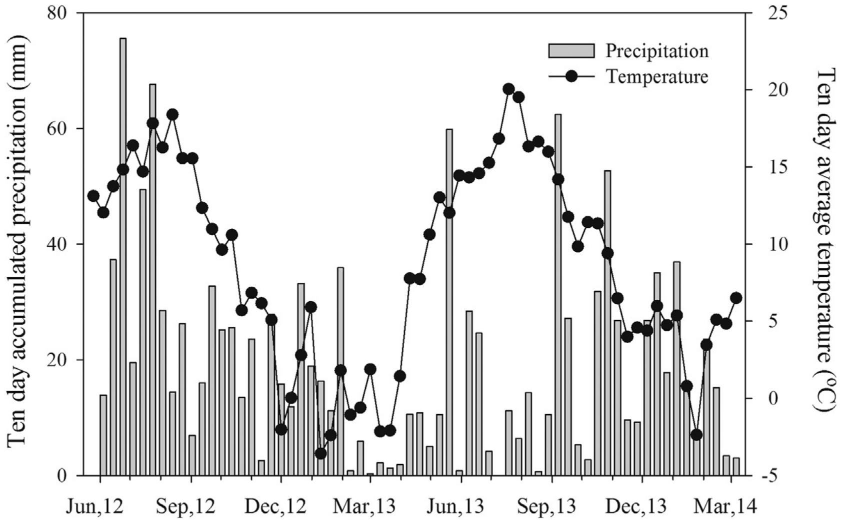

Fig. 1. Ten day average temperature and ten day accumulated precipitation from planting of leeks in 2012 to the last soil sampling in 2014.

total number of roots crossing the lines of each 40 × 40 mm cross (number of root intersections per meter line). The average of all grid crosses within a certain soil layer was calculated as root intensity for the soil layer. The root growth was measured on August 23, September 13, October 8, November 7, 2012 and April 3, 2013 in the S, S_{emp} and IE systems in 2012. In 2013, root growth was measured on August 26, September 10, September 27, October 25, November 26, 2013 and March 13, 2014 in all treatments.

2.3. Soil and plant sampling and analysis

Leek was hand harvested October 24–25, 2012 and September 25, 2013 (two rows of leek × 3 m per plot). The dyer's woad was sampled twice each year after harvest on October 30, 2012 and October 8, 2013, and in late autumn at the time considered as the start of the leaching season on November 26, 2012 and November 28, 2013 (one row × 1 m). Plant samples were sorted into leek and dyer's woad. Residues were removed from the leeks and the marketable yield was evaluated by product size, shape and damage by pests or diseases according to the market standard. Plant and residue samples were chopped, mixed well and oven dried at 80 °C for 48 h. The total plant N was measured by the VDLUFA method (VDLUFA, 1991). First, plant material was burnt at 900 °C and then molecular N was determined by use of LECO TruSpec CN (St. Joseph, Michigan). Soil was sampled at planting (May 24, 2012 and May 14, 2013), harvest (October 30, 2012 and October 1, 2013), in late autumn at the start of the leaching period (November 26, 2012 and November 28, 2013) and in early spring the following year at the end of the leaching period (April 4, 2013 and March 26, 2014). The leaching period was defined as the main period after harvest, where the risk of nitrate leaching is considered to be highest according to climate and soil conditions in Denmark (Jensen et al., 1994). Dyer's woad was not sampled in early spring due to low biomass after winter. For soil sampling, ten replicates were taken in each plot with a 14 mm inner-diameter soil piston auger. Ten soil samples were taken representatively in rows and interrows in each plot. They were separated by depths of 0–0.3 m, 0.3–0.75 m, 0.75–1 m, 1–1.5 m, 1.5–2 m and 2–2.5 m and mixed into a composite sample for each depth and plot. The soil samples were frozen until analysis then thawed and subsamples of 100 g fresh weight were extracted in 1 M KCl for 1 h (1 soil:2 solution). The soil extract was centrifuged and the supernatant was analyzed for NH_4^+ and NO_3^- by standard colorimetric methods using AutoAnalyzer 3 (Bran + Luebbe, Germany).

2.4. Calculation and data analysis

Due to the substitutive design in this experiment, relative competition intensity (RCI) based on relative yield (RY) (Fowler, 1982) was used to study the competition between the crop and catch crop (Williams and McCarthy, 2001), where the proportion of crop in the intercropped system was taken into account. The RCI was calculated as follows:

$$RY_A^D = Y_{AB}^D / \left(p_A Y_A^D\right)$$

$$RCI_A^D = 1 - RY_A^D$$

Where Y_{AB}^D is the yield of crop A in the intercropped system, Y_A^D is the yield of crop A in monoculture and p_A is the proportion at which crop A was sown. In our case, the mean dry biomass of leek in monoculture from three replicates was used. RY_A^D is the relative yield of crop A, calculated as the yield of crop A in mixture divided by the yield of crop A in the sole-cropped system. The superscript D after RY is the density at which plants were grown. D equals to 67 in our case. RCI is used to indicate the existence of competition. An RCI value equal to 0 indicates there is no effect from the competitor. If the value is positive, it indicates the existence of competition. If it is negative, it means no competition with species B.

The root depth penetration rates were estimated using the average root depth at each measurement and the accumulated average daily temperature from sowing to measurement dates (base temperature of 0 °C), following the method put forward by Barraclough and Leigh (1984).

Statistical significance of differences was tested by using the Kenward-Roger based method (R software, version 3.0.2). The treatment effects on yield, dry biomass, aboveground N accumulation and total soil N_{inorg} were tested by using a mixed model analysis of variance (ANOVA) with System (S, IE, IL and S_{emp}), Year (2012 and 2013) and System × Year as fixed factors and Block as random factor. The Year was taken as a fixed factor, because there was one month difference in harvest time between the two years. If the interaction of System and Year was found significant, data were analyzed in separate years. The data of root intensity and soil N_{inorg} in each soil layer were also analyzed in separate years. The homogeneity of variances was tested by Bartlett test. If variances were not homogeneous, data were transformed by the function $y = X^{1/2}$ or $y = \log(x)$ to obtain homogeneity. Tukey's test was applied for multiple pairwise comparisons among treatments. The significant

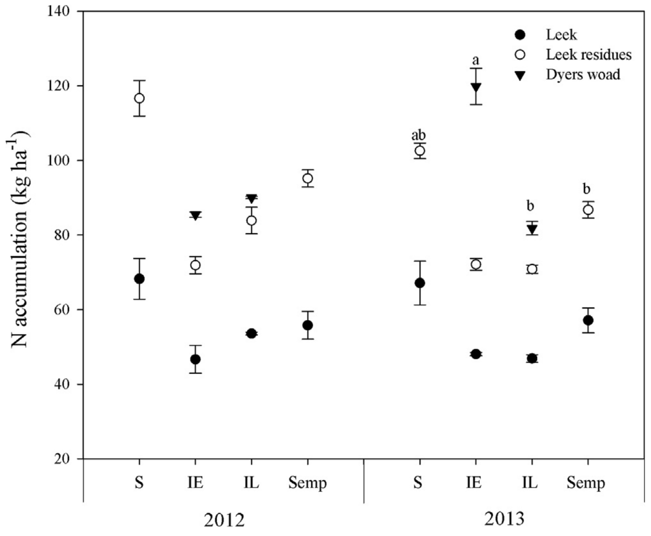

Fig. 2. The N accumulation of plants in four systems at harvest in 2012 and 2013. The abbreviations of S, IE, IL, S_{emp} are explained in Table 1. Different lower case letters indicate differences in the aboveground total N accumulation between systems in separate years at $p < 0.05$. Bars indicate standard error of means.

effects of System, Year and interaction were reported with an asterisk, and results of the pairwise comparisons within each factor were reported with letters.

3. Results

3.1. *Yield of leek and biomass accumulation*

The total yield and dry biomass of leek by area in the S system was higher than the IE and IL systems, while it was similar between the IE and IL systems (Tables 1 and 2). The marketable yield in the S system was similar to the IE system, but was higher than in the IL system ($p < 0.05$). When calculated as kilograms per meter row, the total yield in the S_{emp} system was higher than in the S system.

Interactions were found between year and system in the dry biomass for dyer's woad at harvest and start of the leaching period as well as for RCI values (Table 2). In both years, early sown dyer's woad had higher dry biomass compared to late sown dyer's woad at harvest. The presence of leek decreased the dyer's woad biomass (IE, IL) compared to dyer's woad growing alone at harvest (DE_{emp}, DL_{emp}) and start of the leaching period (DE_{emp}). From harvest to the start of the leaching period, the biomass of dyer's woad decreased, more for early sown than late sown dyer's woad in 2012 ($p < 0.05$). In 2013, the late sown dyer's woad increased the biomass after harvest ($p < 0.05$), but with early sown dyer's woad it stayed the same. The relative competition index (RCI) was negative in all systems, and was lower in the IE than the IL in 2013 (Table 2).

3.2. *N aboveground accumulation*

Significant interaction between year and system was found in total aboveground N accumulation ($p < 0.01$) and N accumulation of dyer's woad ($p < 0.01$). The S system tended to have higher total N accumulation than the IE ($p = 0.05$) and IL ($p = 0.1$) in 2012 (Fig. 2). In 2013, the IE system accumulated most total N, with 48 kg N ha^{-1} in dyer's woad, the total being higher than the IL and S_{emp} systems. The total N accumulation in leek, including leek and leek residues was highest in the S system ($p < 0.05$), and it was higher in the S_{emp} system than in the IE system ($p < 0.05$). Interaction between year and system existed in dyer's woad N accumulation. However, in both years, early sown dyer's woad accumulated more N than late sown dyer's woad at harvest (Fig. 2) ($p < 0.05$) and at the start of the leaching period in 2012 (results not shown). In 2012, N accumulation of early and late sown dyer's woad decreased from harvest to the start of the leaching period, and early sown decreased the most ($p < 0.05$). In 2013 from harvest to the start of the leaching period, the mean aboveground N accumulation of late sown dyer's woad increased (14 kg N ha^{-1}), while it was slightly decreased by 3 kg N ha^{-1} in early sown dyer's woad (results at the start of leaching period not shown).

3.3. *Soil N_{inorg}*

There was interaction between year and system on total soil N_{inorg}, which influenced results at harvest and start of the leaching period (Table 3). In 2013, the IE system had lower total soil N_{inorg} than the IL. It tended to be 52 and 38 kg N ha^{-1} lower than the S system ($p < 0.08$) at harvest and start of the leaching period, respectively. At the end of the leaching period the average of total soil N_{inorg} in the two years was higher in IL (average of 108 kg N ha^{-1}, $p < 0.05$) and S_{emp} (average of 137 kg N ha^{-1}, $p < 0.01$) systems than in the IE system (average of 98 kg N ha^{-1}).

In general, the highest soil N_{inorg} was found in the same soil layer in all systems at each sampling time in both years (Fig. 3). The highest soil N_{inorg} was found in the soil layer of 0.3–0.75 m at harvest, then, in the soil layer of 1.0–1.5 m at start of the leaching period, and in the soil layer of 1.5–2 m at the end of the leaching period. In 2012, the soil N_{inorg} in each soil layer at harvest was not affected by the system, which is accordance with the result of total soil N_{inorg} (Table 3). However, at the start of the leaching period the soil N_{inorg} in the S_{emp} system was higher than in the S and IE systems in the soil layer of 0.3–0.75 m, and at the end of the leaching period was higher than in the IL system in the soil layer of 1.5–2 m (Fig. 3). At harvest time in 2013, the soil N_{inorg} was lower in the IE system than the S_{emp} system in the soil layer of 0.3–0.75 m, and was lowest in the IE system compared to the other systems in the soil layer of 0.75–1 m. At the start of the leaching period, soil N_{inorg} was higher in the IL system than in the IE system in the 0.75–1 m and 1–1.5 m soil layers. In addition, at the start of the leaching period, the soil N_{inorg} below 0.75 m in the IE system was 101 kg N ha^{-1}, which was 33, 62 and 62 kg N ha^{-1} less than the S, IL and S_{emp} systems, respectively ($p < 0.05$). At the end of the leaching period, the IE left 59 kg N ha^{-1} soil N_{inorg} in the soil layer of 0.75–2.5 m, which was less than in the S system (82 kg N ha^{-1}; $p < 0.05$).

3.4. *Root depth and distribution*

The root depth in systems including dyer's woad increased over time (Fig. 4). In 2012, roots in the IE and DE_{emp} extended from 0.5 and 0.4 m to 2.0 and 1.8 m depth and in 2013 from 0.8 and 0.7 m to 2.1 m depth from the first measurement to the start of the leaching period. The deepest roots of late sown dyer's woad (IL, DL_{emp}) were found above 0.25 m at the first measurement and reached to the soil depth of 1.6 and 1.4 m at the start of the leaching period. Roots of late sown dyer's woad (IL, DL_{emp}) in 2013 grew 0.7 m and 0.5 m deeper during the winter time. Apparently IE and DE_{emp} root depth did not increase over winter, but this was due to the limited length of the minirhizotrons being unable to show the changes in root depth below 2.3 m. Using the results from the first measurements until the start of the leaching period and the accumulated daily temperature from sowing, the root depth penetration rate of dyer's woad was found by simple linear regression to be 1.9 mm d^{-1} $^{\circ}C^{-1}$ in 2012 and 1.6 mm d^{-1} $^{\circ}C^{-1}$ in 2013 ($R^2 = 0.96$ for both, $p < 0.01$).

From harvest to the start of the leaching period, the zone where roots were present under the leek row in 2012 extended from the soil layer of 0–1.25 m to 0–2 m in the IE system and from the soil layer of 0–2 m to 0–2.3 m in the DE_{emp} system. During the same

Table 1
The total and marketable yield of leek in four systems in 2012 and 2013.

Factor	Total yield		Marketable yield		Marketable yield of total yield (%)
	Mg ha^{-1}	Kg m^{-1} row	Mg ha^{-1}	Kg m^{-1} row	
§System					
S	38.6^{a}	2.06^{b}	26.7^{a}	1.43	68
IE	28.8^{b}	2.17^{ab}	20.4^{ab}	1.51	71
IL	30.1^{b}	2.41^{ab}	18.0^{b}	1.44	59
S$_{emp}$	31.6^{b}	2.53^{a}	20.5^{ab}	1.64	65
Year					
2012	30.0	2.10	15.8^{b}	1.07^{b}	53
2013	34.6	2.48	27.0^{a}	1.93^{a}	78

§ S: sole-cropped system; IE: intercropped system with early sown dyer's wood; IL: intercropped system with late sown dyer's wood; S$_{emp}$: sole crop with empty rows. Different lower case letters indicate differences between systems or years at $p < 0.05$.

Table 2
The dry biomass of leek and dyer's woad in six systems and the relative competition index (RCI) compared to the sole-cropped system.

	System§	Aboveground biomass (kg ha^{-1})				RCI (leek) (%)
		Leek harvest	Dyer's woad harvest	Total harvest	Dyer's woad start of leaching period	
2012	S	6716^{a}	–	6716^{a}		–
	IE	4787^{b}	279^{b}	5066^{b}	143^{b}	−7
	IL	4960^{b}	121^{c}	5081^{b}	73^{b}	−11
	S$_{emp}$	5411^{b}	–	5411^{b}		–
	DE$_{emp}$		485^{a}		967^{a}	
	DL$_{emp}$		269^{a}		206^{b}	
2013	S	6541^{a}	–	6545^{a}		–
	IE	4966^{b}	1200^{b}	6165^{a}	1297^{b}	−14^{b}
	IL	4556^{b}	379^{d}	4936^{b}	719^{b}	−5^{a}
	S$_{emp}$	4992^{b}	–	5015^{b}		–
	DE$_{emp}$		2133^{a}		1924^{a}	
	DL$_{emp}$		843^{c}		1230^{b}	
	Year	ns				
	System	***				
	Year x System	ns	*	*	*	*

Ns: not significant.

§ The abbreviation of S, IE, IL and S$_{emp}$ are explained in Table 1. DE$_{emp}$: early sown dyer's woad with empty row; DL$_{emp}$: late sown dyer's woad with empty row. Different lower case letters indicate differences at $p < 0.05$, between systems in separate years.

*** Indicate difference of experimental factors at $p < 0.001$ and $p < 0.05$ between systems across years or of interactions between factors.

* Indicate difference of experimental factors at $p < 0.001$ and $p < 0.05$ between systems across years or of interactions between factors.

period the root intensities in the soil layer of 0.5–1.5 m increased in the two systems (Figs. 5 and 6a). Under the dyer's woad row, the root intensity below 1.25 m was higher at start of the leaching period (Fig. 6c) than at harvest (Fig. 5c). In 2013 under the leek row, the rooted zone extended from harvest to the start of the leaching period from the soil layer of 0–0.75 m to 0–2.3 m in the IE and DE$_{emp}$ systems, from the soil layer of 0–0.5 m to 0–1.75 m in the IL system and from the soil layer of 0–0.75 m to 0–1.75 m in the DL$_{emp}$ system, although the amount of roots was small (Figs. 5 and 6b). The root intensities at start of the leaching period (Fig. 6b) were higher than at harvest (Fig. 5b) in the soil layers of 0–0.25 m and 0.25–0.5 m in the IE and IL systems, respectively, and below 0.25 m in the DE$_{emp}$ system, and in the soil layer of 0–1.25 m in the DL$_{emp}$ system. In 2013 under the dyer's woad row, the rooted zone extended from harvest to the start of the leaching period from the soil layer of 0–1.75 m to 0–2.3 m in the IE and DE$_{emp}$ systems and from 0 to 1 m to 0–2 m in the IL and DL$_{emp}$ systems (Figs. 5 and 6d). The root intensity increased in this period below 1 m in the IE and DE$_{emp}$ systems, and in the soil layer of 0.25–1.75 m in the IL and DL$_{emp}$ systems.

The roots of early sown and late sown dyer's woad were only compared in 2013. Early sown dyer's woad had deeper roots than late sown dyer's woad from August until November. The difference in root depth between early sown and late sown dyer's woad ranged from 0.2 m (September 26) to 0.9 m (September 10) ($p < 0.01$). No difference was found in root depth of dyer's woad sown at the same time. Differences in root intensity between systems were first observed at harvest (data from earlier dates not shown). At harvest in 2013, the IE system showed higher root intensity than the IL in the soil layer of 1–1.25 m ($p < 0.05$) and higher than the IL and DL$_{emp}$ in the soil layer of 1.25–1.5 m (Fig. 5d). At the start of the leaching period 2013, the root intensity of the DE$_{emp}$ system was higher than that of DL$_{emp}$ in the soil layer of 1.25–1.5 m ($p < 0.05$), and the intensities of the IE system were higher than those of the IL and DL$_{emp}$ in the soil layer of 1.5–2 m ($p < 0.05$) (Fig. 6d). The root intensities in the soil layer of 1–2.3 m in the dyer's woad row are displayed over time only for the IE, IL, DE$_{emp}$ and DL$_{emp}$ systems (Fig. 7), since only these systems with dyer's woad had roots in the subsoil. Significant difference between systems was found in 2013 at harvest and thereafter. Early sown dyer's woad had higher root intensity than the late sown. No significant difference was observed between systems where dyers woad was sown at the same dates. However, the IL system had double root intensity in the 0.75–1.75 m soil layer than dyer's woad growing alone (DL$_{emp}$) ($p < 0.05$), both at start and end of the leaching period (Fig. 8).

4. Discussion

4.1. The leek yield and competition in sole-cropped and intercropped systems

The interspecific competition between early sown dyer's woad and leek was not strong enough to hamper the leek yield, relative to the intraspecific competition in the sole-cropped system. The

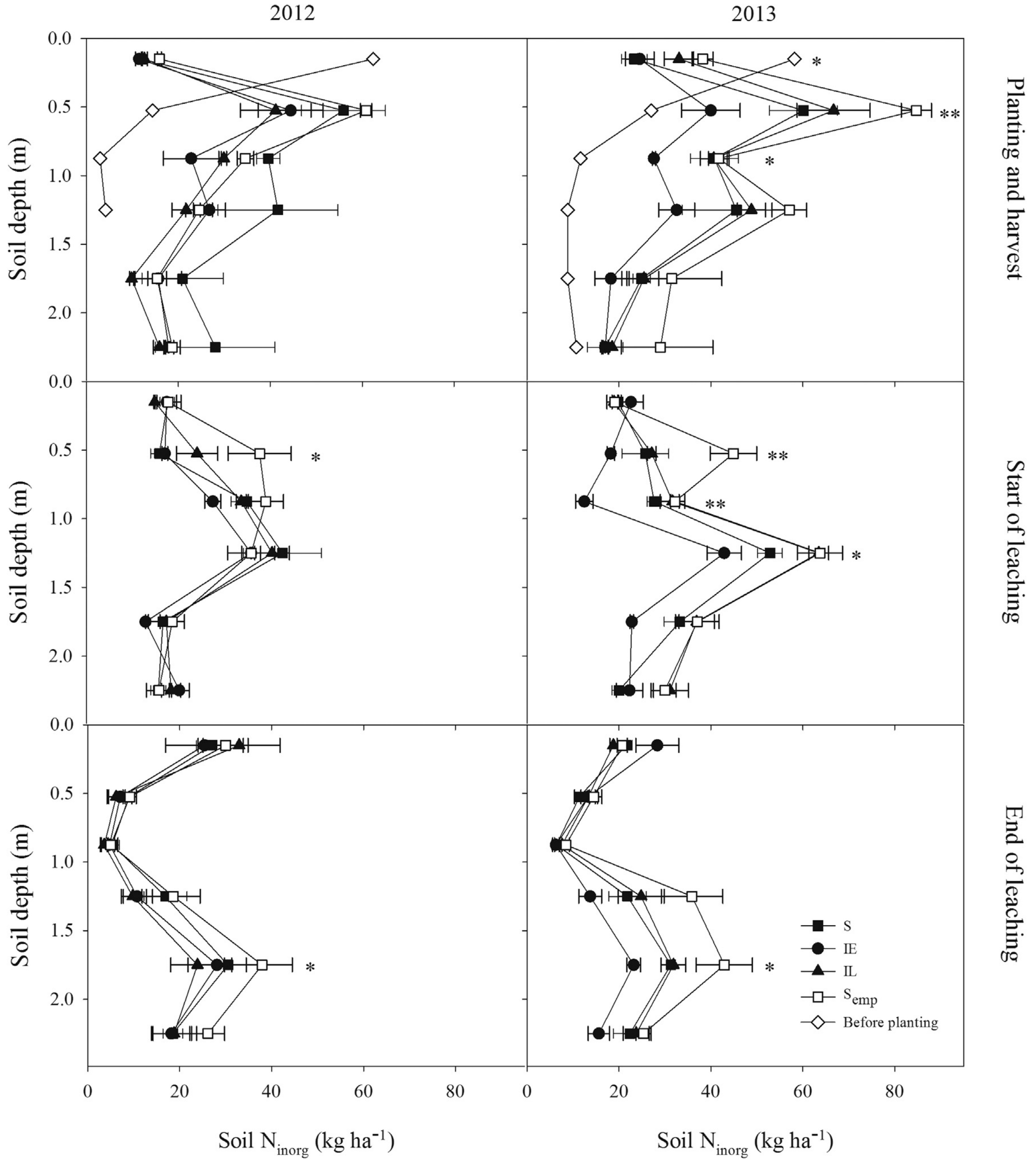

Fig. 3. The soil N_{inorg} distributed in the soil layers of 0–2.5 m at planting, harvest, start of the leaching period and end of the leaching period in 2012 and 2013. The abbreviations of S, IE, IL, S_{emp} are explained in Table 1. The * and ** indicate differences between systems in separate years at $p < 0.05$ and $p < 0.01$, respectively. Bars indicate standard error of means.

total yield of leek per area was lower in the intercropped systems due to lower crop density rather than competition, since the total yield per length of row was similar in the IE, IL and S systems and the RCI values were not positive (Table 2). Thus, a high interspecific competition with early sown dyer's woad in hypothesis 1 was not confirmed. On the other hand, all RCI values in the intercropped systems were negative, which showed an interspecific facilitation of leek (Table 2). Furthermore, the lower total yield by meter row in the S system compared to the S_{emp} system indicated intraspecific competition in the S system. With 67% of leek density in the S system, the total yields of the IE, IL and the S_{emp} systems were 74–83% of the yield in the S system (Table 1). It indicated that the interspecific competition in the intercropped system was not equally high as the intraspecific competition in the sole-cropped system, and the lower crop density in the IE, IL and S_{emp} systems alleviated the intraspecific competition to some extent as found in cauliflower intercropped with overwintering grass-clover (Xie and Kristensen, 2016). Moderate decrease of crop density has shown higher land equivalent ratio (LER) in wheat (*Triticum aestivum* L.) intercropped with beans (*Vicia faba* L.) (Bulson et al., 1997) and achieved higher biomass and yield of sorghum (*Sorghum bicolor* L.) and cowpea (*Vigna unguiculata* L. Walp.) compared to recommended crop density (Karanja et al., 2014). Another reason for the low interspecific competition could be low competitiveness of dyer's woad that has shown low growth response to changes of soil N, and low N requirements (Monaco et al., 2005).

Although the leek density in the IE system was one third lower than the S system, the marketable yield was similar between the IE

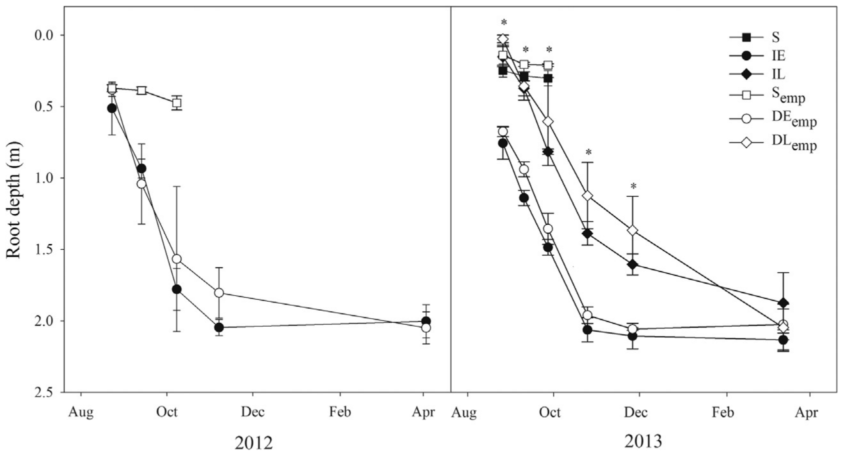

Fig. 4. Root depth development under the leek row in the S and S_{emp} systems and under the dyer's woad row in the other systems in 2012 and 2013. The abbreviations of S, IE, IL, S_{emp}, DE_{emp} and DL_{emp} are explained in Table 1 and Table 2. The * indicates differences between systems in separate years at $p < 0.05$. Bars indicate standard error of means.

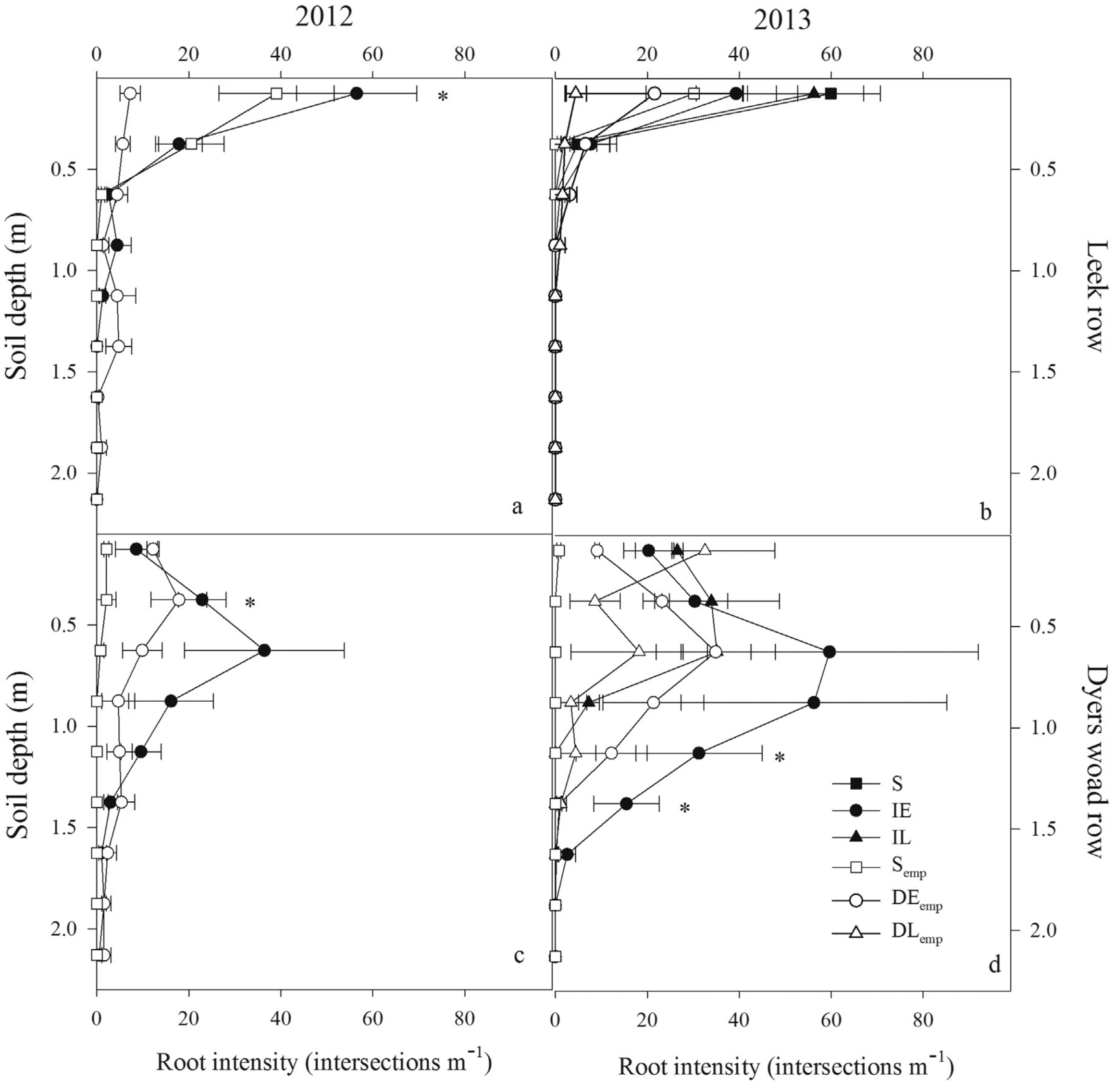

Fig. 5. Root intensity in the soil layer of 0–2.3 m at harvest. a and b: The root intensity under the leek row in S, IE, IL and S_{emp} or empty row in DE_{emp} and DL_{emp} in 2012 and 2013, respectively. c and d: The root intensity under the dyer's woad row in S_{emp}, IE, IL, DE_{emp} and DL_{emp} in 2012 and 2013, respectively. The abbreviations of S, IE, IL, S_{emp}, DE_{emp} and DL_{emp} are explained in Table 1 and Table 2. The * indicates differences between systems in separate years at $p < 0.05$. Bars indicate standard error of means.

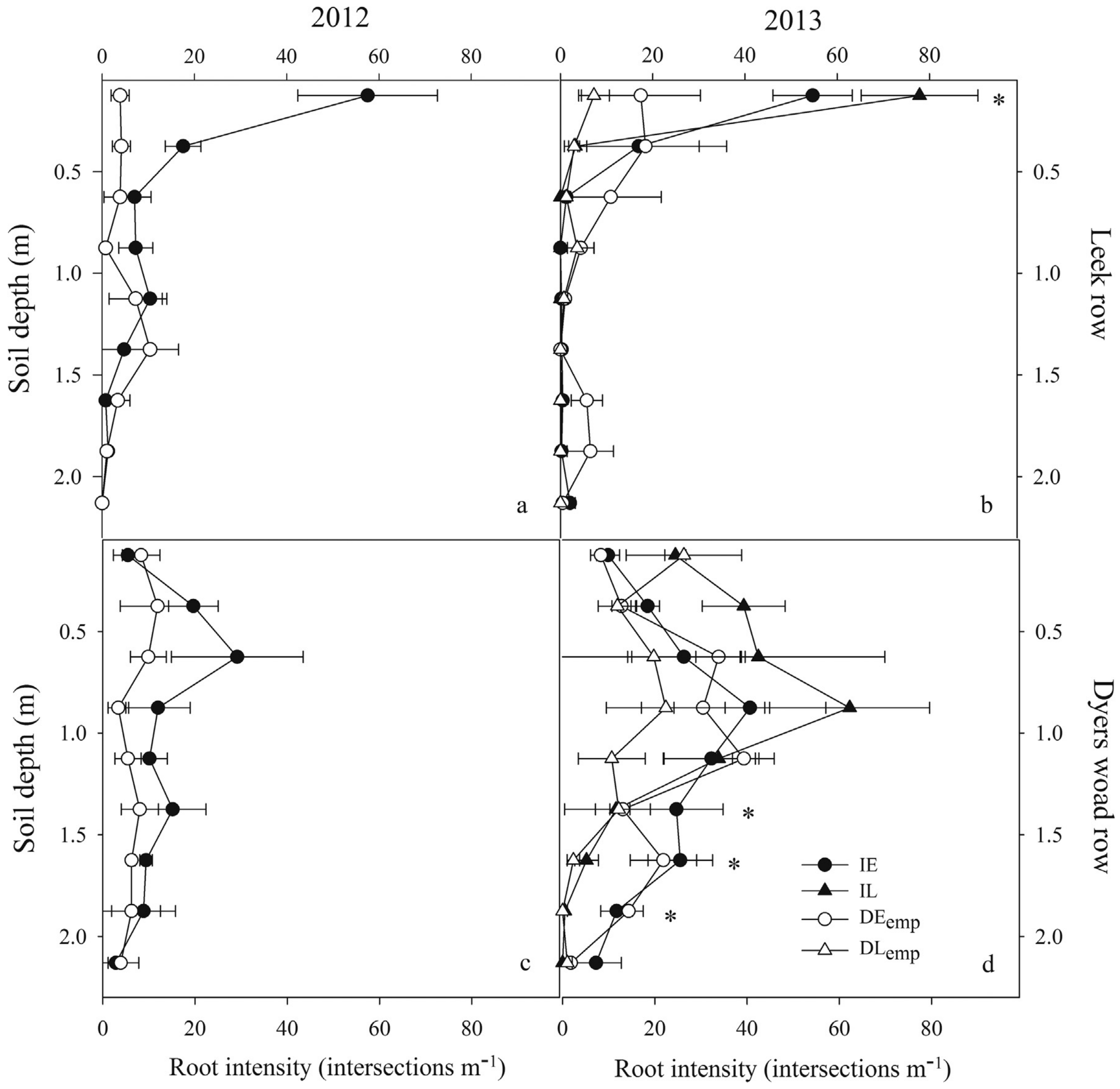

Fig. 6. Root intensity in the soil layer of 0–2.3 m at the start of the leaching period. a and b: The root intensity under the leek row in IE and IL or empty row in DE_{emp} and DL_{emp} in 2012 and 2013, respectively. c and d: The root intensity under the dyer's woad row in IE, IL, DE_{emp} and DL_{emp} in 2012 and 2013, respectively. The abbreviations of IE, IL, DE_{emp} and DL_{emp} are explained in Table 1 and Table 2. The * indicates differences between systems in separate years at $p < 0.05$. Bars indicate standard error of means.

Table 3
The soil N_{inorg} in four systems at planting, harvest, start of the leaching period and end of the leaching period in the 0–2.5 m soil layer.

Year	System[§]	Total soil inorganic N (kg ha^{-1})			
		Before planting	Harvest	Start of leaching	End of leaching
2012	S	[†]84.5	198	143	107
	IE		139	131	94
	IL		131	149	95
	S_{emp}		170	165	127
2013	S	125.4	212^{bc}	179^{ab}	115
	IE		160^{c}	141^{b}	105
	IL		233^{ab}	209^{a}	120
	S_{emp}		282^{a}	227^{a}	148
	Year				ns
	System				*
	Year × System		*	*	ns

[§] The abbreviations of S, IE, IL and S_{emp} are explained in Table 1.

[†] The soil N_{inorg} was only measured in the 0–1.5 m soil layer before planting. Lower case letters indicate the difference between systems in separate years at $p < 0.05$.

[*] Indicates significant difference between systems across years or significant interaction between factors at $p < 0.05$. Ns: not significant.

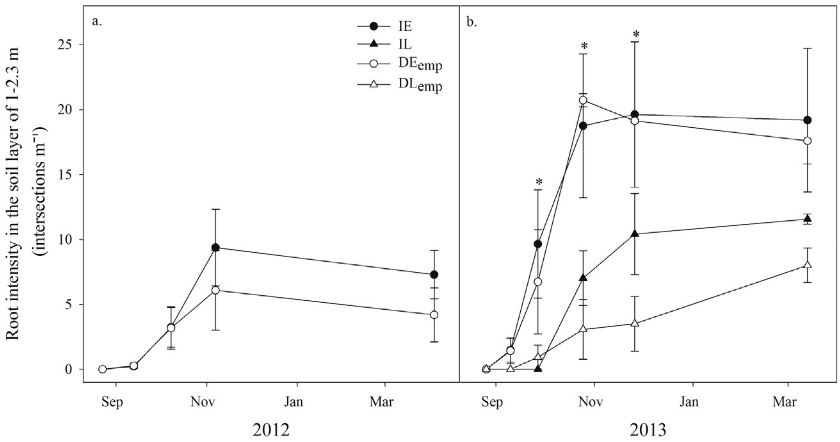

Fig. 7. Mean root intensity in the soil layer of 1–2.3 m under the dyer's woad row in IE, IL, DE$_{emp}$ and DL$_{emp}$. The abbreviation of IE, IL, DE$_{emp}$ and DL$_{emp}$ are explained in Table 1 and Table 2. The * indicates differences between treatments in separate years at $p < 0.05$. Bars indicate standard error of means.

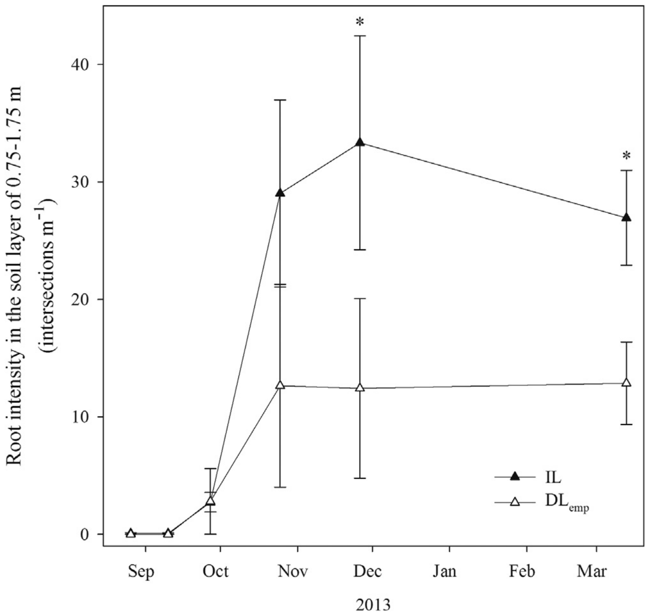

Fig. 8. Mean root intensity in the soil layer of 0.75–1.75 m under the late sown dyer's woad row in IL and DL$_{emp}$ systems. The abbreviations of IL and DL$_{emp}$ are explained in Table 1 and Table 2. The * indicates differences between systems at $p < 0.05$. Bars indicate standard error of means.

and S systems (Table 1). This seemed to be due not to a decreased incidence of pest and diseases due to intercropping with dyer's woad as reported in Canali et al. (2016), although catch crops have been reported to decrease pest populations (Depalo et al., 2016; Masiunas, 1998). The marketable yield of the two intercropped systems was, like the total yield, higher or close to 67% of the marketable yield of leek in the S system due to reduced leek density. Therefore such intercropped systems with substitutive design will decrease farmers' income per area. However, considering the similar marketable yield per length of row in all systems, this can be compensated for by increasing the vegetable growing area if other benefits from agro-ecological services are achieved (Kremen and Miles, 2012).

Late sowing of dyer's woad 8 weeks after leek transplanting did not show positive effect on the competiveness and yield of leek, compared to early sown dyer's woad. The delay of dyer's woad sowing for 5 weeks worked well to control the competitiveness to leek, according to the similar leek yields between S and IE calculated per length of row. Similar result has been shown in a 6-week delayed sowing of ryegrass in leek, while a 4-week delay (Müller-Schärer, 1996) or 2–4 day delay (Munkholm and Hansen, 2012) caused severe yield reduction. Additionally, the lower RCI value in the IE system than in the IL system in 2013 indicated that dyer's woad in the IE system stimulated growth of leek (Table 2). Wang et al. (2009) showed that it was possible to increase the yield of onion and celery with crucifer biofumigants by enhancing beneficial microorganisms. Also allelopathic water extract of cruficers can improve crop growth at low concentration (Farooq et al., 2013). This stimulation of leek growth and low interspecific competition relative to intraspecific competition did not support hypothesis 1 on the need to delay sowing to 8 weeks to achieve similar yields of leek compared to the sole-cropped system. On the other hand, the competition jeopardized the dry biomass of dyer's woad (Table 2), compared to dyer's woad grown with empty rows.

Dyer's woad did not continue aboveground growth after harvest of the leek, except for late sown dyer's woad in 2013. Due to the longer growing period, early sown dyer's woad had higher dry biomass than late sown dyer's woad (Table 3). Year and cropping system had a combined effect on dyer's woad biomass at harvest and start of the leaching period. The decrease of biomass from harvest to the start of the leaching period in 2012 could be attributed to a shorter growth period before entering the winter dormant stage due to the harvest being one month later than in 2013. In 2013, the increase of biomass of late sown dyer's woad could be attributed to a more vigorous growth stage compared to the early sown dyer's woad.

4.2. *Complementary root systems and root growth in the intercropped systems*

Early sown dyer's woad had a deeper and denser root distribution than late sown dyer's woad before the start of the leaching period, which confirms hypothesis 1 that late sown dyer's woad has fewer roots, compared to early sown dyer's woad. The root depth of annual crops is closely related to accumulated temperature (Kristensen and Thorup-Kristensen, 2004). This relationship was also found in the present study and explains the difference of root depth between early sown and late sown dyer's woad before the start of the leaching period. Consistent with root depth, the root intensity in the subsoil layer (mainly 1–1.5 m) in the IE system was also higher than in the IL (Figs. Fig. 5d and Fig. 7b). The significant difference moved from the 1–1.5 m soil layer at harvest to the 1.25–2 m soil layer at the start of the leaching period.

Both early and late sown dyer's woad developed root systems below 2 m by the end of the experiment, which was comparable to the results of Munkholm and Hansen (2012) and Thorup-Kristensen and Rasmussen (2015). The highest root intensity in the present study was found in the soil layer of 0.5–0.75 m at harvest and then decreased with soil depth (Fig. 5d). In a field experiment where dyer's woad was undersown in spring barley (*Hordeum vulgare* L.), the highest root intensity was in the top soil layer of 0–0.5 m, and in the soil layer of 0.5–0.75 m the root intensity was around 30 intersections m^{-1} at barley harvest (Thorup-Kristensen and Rasmussen, 2015), which was lower than in the present study (60 intersections m^{-1}). This could be the result of strong interspecific competition, which reduced deep root growth of both barley and dyer's woad. The root depth penetration rate of dyer's woad in the present study was 1.6–1.9 mm d^{-1} °C^{-1} in accordance with the fast root development found in other crucifers such as white cabbage and Chinese cabbage (Kristensen and Thorup-Kristensen, 2007), but lower than fodder radish of 3.5 mm d^{-1} °C^{-1} (Kristensen and Thorup-Kristensen, 2004).

Dyer's woad increased the root zone of the whole cropping system by its deep root system and by extending roots to the neighboring row, confirming hypothesis 2 that intercropping dyer's woad increases the soil volume explored by roots compared to the sole-cropped system. Leek roots were distributed in the soil layer of 0–0.5 m, mainly in the soil layer of 0–0.25 m as shown in the S and S_{emp} systems (Fig. 5a and b), which corresponds well with previous results (Kristensen and Thorup-Kristensen, 2007). In addition, leek roots were mainly confined in the leek row instead of spreading into the neighboring row, since the S_{emp} system had very few roots under the empty row (Fig. 5c and d). With such a confined root system of leek, the intercropped systems increased the root exploring area of the whole cropping system, since the dyer's woad distributed roots into the 0–2 m soil layer, beyond the leek root zone (Figs. 5c, d and 6c, d). Schröder and Köpke (2012) also reported that intercropped systems increased the root-length density in the soil layer of 0.12–0.6 m. Moreover, in 2012, dyer's woad roots extended underneath the root zone of the neighboring leek row in the soil layer of 0.5–1.25 m at harvest (Fig. 5a) and 0.5–2 m at the start of the leaching period (Fig. 6a). Similar results have been also reported in other field studies. For example, intercropped wheat (*Triticum aestivum* L.) extended its roots under maize (*Zea mays* L.) in the measured depth of 0–0.6 m (Li et al., 2006). When intercropped with soybeans (*Glycine max* L. Merr.) under full irrigation, maize extended roots into the root zone of two neighboring adjacent soybean rows with a row distance of 30 cm, mainly observed in the soil layer of 0.16–0.22 m (Gao et al., 2010). However, the root growth pattern in 2013 was different from in 2012. In 2013, hardly any roots of dyer's woad were found under the leek row in the soil layer below 0.5 m (Figs. 5b and 6b), but the root intensity in the dyer's woad row was double that in 2012 in the soil layer of 1–2.3 m (Fig. 6c and d). This might be related to a lower accumulated precipitation from sowing to the third root measurement (2012: 280 mm; 2013: 133 mm) stimulating deep root growth for water absorption in 2013. Another possible reason could be the different soil N_{inorg} in the soil layer of 0.75–1.5 m before planting (7.5 and 20.5 kg N ha^{-1} in 2012 and 2013, respectively), which may be a result of different pre-crops in two years. The higher soil N_{inorg} in the deep soil layer in 2013 might have stimulated deep root growth as found by Kristensen and Thorup-Kristensen (2007).

Only the DE_{emp} system in 2013 (Fig. 6b) had roots in the soil layer of 1.5–2 m under the empty leek row, and it appears that the interaction between the two plant species played a role in the different root growth patterns between years. Root distribution was also found to be changed by the presence of neighboring plants in other studies, where barley intercropped with pea had a faster and deeper root distribution and maize intercropped with wheat or faba bean (*Vicia faba* L.) had a greater root length density in comparison with sole-cropped, probably due to the interspecific competition for nutrients (Hauggaard-Nielsen et al., 2001; Li et al., 2006).

Continued root growth of dyer's woad was observed after harvest (Fig. 5c, d, 6c, d and 7b), which confirmed the hypothesis 4 that dyer's woad kept growing after leek harvest, although aboveground growth was only found in late sown dyer's woad in 2013. The continued root growth during winter was confirmed by the increase of root depth of the late sown dyer's woad after the start of the leaching period. This could not be confirmed for early sown dyer's woad due to the limited measuring depth of 2.3 m of minirhizotrons. The increase of root intensity was observed in both early and late sown dyer's woad from harvest to the start of the leaching period. However, dyer's woad in the two intercropped systems showed different root growth patterns. The root intensity of early sown dyer's woad was increased in the soil layer below 1 m in both years. On the other hand, the root intensity of late sown dyer's woad was increased in almost all soil layers from harvest to the start of the leaching period. The continuous root growth of dyers woad after the harvest below 0.8 m was also found by Thorup-Kristensen and Rasmussen (2015).

Late sown dyer's woad, but not early sown, developed higher root intensities in the IL system compared to the DL_{emp} system before the start of leaching period (Figs. 7b and Fig. 8), which confirms hypothesis 5 that intercropped dyer's woad develops more deep roots than when grown alone. However, the root depth was not influenced. This could be the result of lower soil N_{inorg} at harvest in the root zone (0–1 m) (IL: 140 kg N ha^{-1}, Fig. 3; DL_{emp}: 176 kg N ha^{-1}, data not shown) due to the presence of leek stimulating the deep root development of plants for meeting N demand. This is supported by the lower biomass (Table 2) and N concentration of dyer's woad (IL: 3.0%; DL_{emp}: 3.6%, data not shown) ($p < 0.05$) at harvest in the IL system, compared to the DL_{emp} system. Root growth in subsoil of cabbages and wheat was also denser when N distribution was deep or fertilization was low (Kristensen and Thorup-Kristensen, 2007; Svoboda and Haberle, 2006).

4.3. *The effect of intercropped systems on nitrate leaching and N accumulation*

The N accumulation of dyer's woad and corresponding reduction of soil N_{inorg} of dyer's woad in the IE system showed its potential as an intercrop for N recycling without yield reduction calculated per length of row. The ability to reduce nitrate leaching during the growing season was demonstrated by the differences in total soil N_{inorg} between the IE and the S systems of 59 and 52 kg N ha^{-1} at the harvest in 2012 and 2013, respectively, in accordance with hypothesis 3 that introducing dyer's woad can reduce the risk of nitrate leaching. The difference between the IE and S systems was only significant in 2013, probably due to the higher N accumulation of dyer's woad (Table 2 and Fig. 2). In 2013, the difference was significant in the 0.75–1 m soil layer when the IE system was compared with the S system (Fig. 3). At the start of the leaching period in 2013, the difference was smaller probably due to leaching after harvest especially in the systems with high amounts of soil N_{inorg}, but still the IE system had lower soil N_{inorg} than the IL ($p < 0.05$). Most importantly, the lowest soil N_{inorg} in the IE system (33 and 62 kg ha^{-1} lower than the S and IL, respectively) in 2013 was found in the deep soil layer of 0.75–2.5 m ($p < 0.05$), where N_{inorg} is at highest risk of leaching. The soil N_{inorg} in the IE system was only 1–15 kg ha^{-1} lower than the S and IL systems at the end of the leaching period, which means that a large amount of N might have been lost from harvest to the next spring in the S system (91 and 97 kg ha^{-1} in 2012 and 2013) and in the IL system (113 kg ha^{-1} in 2013), while only 45 and 55 kg N ha^{-1} in the IE system, when assuming other changes by mineralization or denitrification to be minimal or equal in the systems. This demonstrated the role of the IE system in reducing nitrate leaching during the growing season and until the end of the leaching season. This confirmed the hypothesis 4 that dyer's woad keeps taking up soil N_{inorg} after harvest. This lower leaching in the IE system could result from the dense and deep root system being active in N uptake after harvest. Correspondingly, root systems without leek shoots in the S system (results not shown) and with more shallow distributed roots in the IL system (Fig. 6) were observed from harvest until the start of the leaching period, which explains the higher leaching in these systems. This emphasizes the importance of active roots in subsoils for reduction of soil N losses in agroecosystems. The root system of the shallow-rooted crop of leek was complemented by the deep root system of dyer's woad reducing the risk of leaching compared to the S system. By complementing the poor root system of faba bean with deep root systems of safflower and white mustard, soil N_{inorg} was reduced effectively by enhanced root length density and more homogeneous root distribution in 0–0.6 m depth (Schröder and Köpke, 2012). In other field research, intercropping decreased the nitrate leaching by 17–35 kg N ha^{-1} compared to sole-cropped leek or cauliflower across European conditions (Xie et al., 2016), and nitrate leaching was reduced by 15–37% when maize was intercropped with red fescue (*Festuca rubra* L.) (Manevski et al., 2014).

Late sowing decreased the ability of dyer's woad to reduce soil N_{inorg} compared to early sowing. This is consistent with hypothesis 3 that delayed sowing reduces the effect of dyer's woad on reduction of the risk of nitrate leaching. In 2013, the soil N_{inorg} in the IL systems was found to be 73 kg ha^{-1} higher than in the IE system at harvest, 68 kg ha^{-1} higher at the start of the leaching period mainly due to a 43 kg N ha^{-1} difference in the soil layer of 1–2.5 m, and higher at the end of the leaching period across years (Table 3 and Fig. 3).

The two intercropped systems did not accumulate more total N aboveground than the sole-cropped. Still dyer's woad in the IE system was able to accumulate the considerable amount of 48 kg N ha^{-1} in 2013 (Fig. 2). At the start of the leaching period the N accumulation was 44 kg N ha^{-1} (data not shown), comparable to the result of around 35 kg N ha^{-1} in the aboveground biomass of dyer's woad reported by (Thorup-Kristensen and Rasmussen, 2015). The higher N accumulation by early sown dyer's woad relative to late sown could be attributed to the longer growing period (Jeranyama et al., 1998). However, no more N was accumulated aboveground after harvest, probably due to senescence (biomass decreased in 2012) or N translocation to roots for overwintering, as shown by a decrease of biomass N concentration (2013: 4.0% at harvest, 3.4% at the start of the leaching period, $p < 0.05$), as found in other perennials (Scagel et al., 2007; Sturite et al., 2006). In addition, Thorup-Kristensen and Rasmussen (2015) found the root/shoot N ratio of dyer's woad in November could be as high as 2.0, indicating that the N-sink capacity of dyer's woad is underestimated if only N accumulation aboveground is measured. Thus the amount of N retained in the aboveground and belowground biomass will be mineralized and increase N availability for the following year's crop, instead of risking to be leached out of the root zone and lost. Moreover, even though leek had higher N accumulation in the S system due to the larger planting area, it produced more leek residue, which accounted for more than 34–40% of N accumulation. The N in leek residue is destined to be discarded at harvest or left in the field to be mineralized. Neither of the two procedures improves the N retention in the agroecosystem. With the same leek density as in the S_{emp} system, the IE system influenced leek N accumulation, which could be due to the interspecific competition for N. This corresponds with the higher N concentration of leek and leek residue (S_{emp}: 1.58% and 2.13%; IE: 1.36% and 1.79%) (data not shown) and higher soil N_{inorg} in the S_{emp} system (Fig. 3 and Table 3), compared to the IE system. However, the total N accumulation was still higher in the IE than in the S_{emp} system, due to dyer's woad N accumulation.

Due to less growth of late sown dyer's woad as seen from the biomass and root distribution compared to early sown dyer's woad, the N accumulation of late sown dyer's woad was 37 kg N ha^{-1} lower, which also resulted in lower total N accumulation (Fig. 2). The increase of dry biomass of late sown dyer's woad from harvest to the start of the leaching period indicated a demand for N during this period, although the increased N accumulation in late sown dyer's woad could not compensate for the difference caused by sowing dates (Table 2).

5. Conclusions

In organic production, dyer's woad intercropped in a substitutive design gave acceptable marketable yields of leek despite a decrease in crop density. Interspecific interactions stimulated or had no effect on the growth of leek but hampered that of dyer's woad. Therefore no benefits were achieved for N uptake by delaying the sowing time of dyer's woad to 8 weeks compared to 5 weeks relative to leek transplanting. Early sown dyer's woad had good potential to reduce the risk of nitrate leaching due to its ability for N retention and a fast growing root system to a deep depth. Dyer's woad increased the rooted zone in the cropping system markedly, in 2012 by extending roots below the neighboring leek row, and in 2013 by retaining N left by the leek. The intercropped system affected the root development of late sown dyer's woad after harvest, which developed more roots in the 0.75–1.75 m soil layer. In conclusion, intercropping with early sown dyer's woad is an applicable tool to reduce nitrate leaching in organic leek production. Given that interspecific competition had only negative effects on dyer's woad rather than leek, it is recommended that dyer's woad could be used as a catch crop in other designs for intercropped or undersown cropping systems.

Acknowledgements

This study was a part of the InterVeg research project (CORE Organic II ERA-NET and GUDP Ministry of Environment and Food of Denmark). We thank Kristian Kristensen, Department of Agroecology, Aarhus University for his valuable assistance on statistical analysis and Astrid Bergmann, Knud Erik Pedersen, Jens Elkjær, Jens Barfod and Bjarne Egedal for their skilled field work.

References

Båth, B., Kristensen, H.L., Thorup-Kristensen, K., 2008. Root pruning reduces root competition and increases crop growth in a living mulch cropping system. J. Plant Interact. 3, 211–221, http://dx.doi.org/10.1080/17429140801975161.

Barraclough, P.B., Leigh, R.A., 1984. The growth and activity of winter wheat roots in the field: the effect of sowing date and soil type on root growth of high-yielding crops. J. Agric. Sci. 103, 59–74, http://dx.doi.org/10.1017/S002185960004332X.

Bulson, H.A.J., Snaydon, R.W., Stopes, C.E., 1997. Effects of plant density on intercropped wheat and field beans in an organic farming system. J. Agric. Sci. 128, 59–71.

Canali, S., Ortolani, L., Campanelli, G., Bavec, M., von Fragstein, P., D'Oppido, D., Kristensen, H.L., 2016. Yield, product quality and energy use in organic vegetable living mulch cropping systems: research evidence and farmers' perception. Renew. Agric. Food Syst. FirstView, 1–14, http://dx.doi.org/10.1017/s1742170516000314.

Depalo, L., Burgio, G., von Fragstein, P., Kristensen, H.L., Bavec, M., Robačer, M., Campanelli, G., Canali, S., 2016. Impact of living mulch on arthropod fauna: analysis of pest and beneficial dynamics on organic cauliflower (*Brassica oleracea* L. var. *botrytis*) in different European scenarios. Renew. Agric. Food Syst. FirstView, 1–8, http://dx.doi.org/10.1017/s1742170516000156.

Dewey, S.A., Price, K.P., Ramsey, D., 1991. Satellite remote sensing to predict potential distribution of dyers woad (*Isatis tinctoria*). Weed Technol. 5, 479–484.

Ebrahimi, E., Bodner, G., Kaul, H.P., Nassab, A.D.M., 2014. Effects of water supply on root traits and biological yield of Durum (*Triticum durum* Desf.) and Khorasan (*Triticum turanicum* Jakubz) wheat. Plant Biosyst. 148, 1009–1015, http://dx.doi.org/10.1080/11263504.2013.850120.

Farooq, M., Bajwa, A.A., Cheema, S.A., Cheema, Z.A., 2013. Application of allelopathy in crop production. Int. J. Agric. Biol. 15, 1367–1378.

Fowler, N., 1982. Competition and coexistence in a north Carolina grassland: III. mixtures of component species. J. Ecol. 70, 77–92, http://dx.doi.org/10.2307/2259865.

Gao, Y., Duan, A., Qiu, X., Liu, Z., Sun, J., Zhang, J., Wang, H., 2010. Distribution of roots and root length density in a maize/soybean strip intercropping system. Agric. Water Manage. 98, 199–212, http://dx.doi.org/10.1016/j.agwat.2010.08.021.

Hauggaard-Nielsen, H., Ambus, P., Jensen, E.S., 2001. Temporal and spatial distribution of roots and competition for nitrogen in pea-barley intercrops – a field study employing ^{32}P technique. Plant Soil 236, 63–74, http://dx.doi.org/10.1023/a:1011909414400.

Jensen, C., Stougaard, B., Østergaard, H.S., 1994. Simulation of nitrogen dynamics in farmland areas of Denmark (1989–1993). Soil Use Manage. 10, 111–118, http://dx.doi.org/10.1111/j.1475-2743.1994.tb00470.x.

Jeranyama, P., Hesterman, O.B., Sheaffer, C.C., 1998. Medic planting date effect on dry matter and nitrogen accumulation when clear-seeded or intercropped with corn. Agron. J. 90, 616–622, http://dx.doi.org/10.2134/agronj1998.00021962009000050009x.

Kang, L., Yue, S., Li, S., 2014. Effects of phosphorus application in different soil layers on root growth, yield, and water-use efficiency of winter wheat grown under semi-arid conditions. J. Integr. Agric. 13, 2028–2039, http://dx.doi.org/10.1016/S2095-3119(14)60751-6.

Karanja, S.M., Kibe, A.M., Karogo, P.N., Mwangi, M., 2014. Effects of intercrop population density and row orientation on growth and yields of sorghum – cowpea cropping systems in semi arid Rongai, Kenya. J. Agric. Sci. 6, http://dx.doi.org/10.5539/jas.v6n5p34.

Kremen, C., Miles, A., 2012. Ecosystem services in biologically diversified versus conventional farming systems: benefits, externalities, and trade-offs. Ecol. Soc. 17, 40, http://dx.doi.org/10.5751/Es-05035-170440 (Artn 40).

Kristensen, H.L., Thorup-Kristensen, K., 2004. Root growth and nitrate uptake of three different catch crops in deep soil layers. Soil Sci. Soc. Am. J. 68, 529–537.

Kristensen, H.L., Thorup-Kristensen, K., 2007. Effects of vertical distribution of soil inorganic nitrogen on root growth and subsequent nitrogen uptake by field vegetable crops. Soil Use Manage. 23, 338–347, http://dx.doi.org/10.1111/j.1473-2743.2007.00105.x.

Li, L., Sun, J.H., Zhang, F.S., Guo, T.W., Bao, X.G., Smith, F.A., Smith, S.E., 2006. Root distribution and interactions between intercropped species. Oecologia 147, 280–290, http://dx.doi.org/10.1007/s00442-005-0256-4.

Müller-Schärer, H., 1996. Interplanting ryegrass in winter leek: effect on weed control, crop yield and allocation of N-fertiliser. Crop Prot. 15, 641–648, http://dx.doi.org/10.1016/0261-2194(96)00033-6.

Manevski, K., Børgesen, C.D., Andersen, M.N., Kristensen, I.S., 2014. Reduced nitrogen leaching by intercropping maize with red fescue on sandy soils in North Europe: a combined field and modeling study. Plant Soil 388, 67–85, http://dx.doi.org/10.1007/s11104-014-2311-6.

Masiunas, J.B., 1998. Production of vegetables using cover crop and living mulches — a review. J. Veg. Crop Prod. 4, 11–31, http://dx.doi.org/10.1300/J068v04n01_03.

Monaco, T.A., Johnson, D.A., Creech, J.E., 2005. Morphological and physiological responses of the invasive weed *Isatis tinctoria* to contrasting light, soil-nitrogen and water. Weed Res. 45, 460–466, http://dx.doi.org/10.1111/j.1365-3180.2005.00480.x.

Munkholm, L.J., Hansen, E.M., 2012. Catch crop biomass production, nitrogen uptake and root development under different tillage systems. Soil Use Manage. 28, 517–529, http://dx.doi.org/10.1111/Sum.12001.

Scagel, C.F., Bi, G.H., Fuchigami, L.H., Regan, R.P., 2007. Seasonal variation in growth, nitrogen uptake and allocation by container-grown evergreen and deciduous rhododendron cultivars. Hortscience 42, 1440–1449.

Schröder, D., Köpke, U., 2012. Faba bean (*Vicia faba* L.) intercropped with oil crops — a strategy to enhance rooting density and to optimize nitrogen use and grain production? Field Crop Res. 135, 74–81, http://dx.doi.org/10.1016/j.fcr.2012.07.007.

Sturite, I., Uleberg, M.V., Henriksen, T.M., Jørgensen, M., Bakken, A.K., Breland, T.A., 2006. Accumulation and loss of nitrogen in white clover (*Trifolium repens* L.) plant organs as affected by defoliation regime on two sites in Norway. Plant Soil 282, 165–182, http://dx.doi.org/10.1007/s11104-005-5697-3.

Svoboda, P., Haberle, J., 2006. The effect of nitrogen fertilization on root distribution of winter wheat. Plant Soil Environ. 52, 308–313.

Theriault, F., Stewart, K.A., Seguin, P., 2009. Use of perennial legumes living mulches and green manures for the fertilization of organic broccoli. Int. J. Veg. Sci. 15, 142–157, http://dx.doi.org/10.1080/19315260802598896.

Thorup-Kristensen, K., Rasmussen, C.R., 2015. Identifying new deep-rooted plant species suitable as undersown nitrogen catch crops. J. Soil Water Conserv. 70, 399–409, http://dx.doi.org/10.2489/jswc.70.6.399.

Thorup-Kristensen, K., Dresboll, D.B., Kristensen, H.L., 2012. Crop yield, root growth, and nutrient dynamics in a conventional and three organic cropping systems with different levels of external inputs and N re-cycling through fertility building crops. Eur. J. Agron. 37, 66–82, http://dx.doi.org/10.1016/j.eja.2011.11.004.

Thorup-Kristensen, K., 1994. The effect of nitrogen catch crop species on the nitrogen nutrition of succeeding crops. Fert. Res. 37, 227–234, http://dx.doi.org/10.1007/bf00748941.

Tuulos, A., Yli-Halla, M., Stoddard, F., Mäkelä, P., 2015. Winter turnip rape as a soil N scavenging catch crop in a cool humid climate. Agron. Sustain. Dev. 35, 359–366, http://dx.doi.org/10.1007/s13593-014-0229-2.

VDLUFA, 1991. Bestimmung Von Leicht löslichen (pflanzenverfügbaren) Haupt-Und Spurennährstoffen, Band I, Die Untersuchung Von Böden, fourth ed. Darmstadt, Germany.

Vanek, S., Wien, H.C., Rangarajan, A., 2005. Time of interseeding of lana vetch and winter rye cover strips determines competitive impact on pumpkins grown using organic practices. Hortscience 40, 1716–1722.

Wang, G., Ngouajio, M., Charles, K.S., 2009. Brassica biofumigants improve onion (*Allium cepa* L.) and celery (*Apium graveolens* (*Mill.*) *Pers.*) production systems. J. Sustain. Agric. 34, 2–14, http://dx.doi.org/10.1080/10440040903396516.

Williams, A.C., McCarthy, B.C., 2001. A new index of interspecific competition for replacement and additive designs. Ecol. Res. 16, 29–40, http://dx.doi.org/10.1046/j.1440-1703.2001.00368.x.

Wyland, L.J., Jackson, L.E., Chaney, W.E., Klonsky, K., Koike, S.T., Kimple, B., 1996. Winter cover crops in a vegetable cropping system: impacts on nitrate leaching, soil water, crop yield, pests and management costs. Agric. Ecosyst. Environ. 59, 1–17, http://dx.doi.org/10.1016/0167-8809(96)01048-1.

Xie, Y., Kristensen, H.L., 2016. Overwintering grass-clover as intercrop and moderately reduced nitrogen fertilization maintain yield and reduce the risk of nitrate leaching in an organic cauliflower (*Brassica oleracea* L. var. *botrytis*) agroecosystem. Sci. Hortic. 206, 71–79.

Xie, Y., Tittarelli, F., von Fragstein, P., Bavec, M., Canali, S., Kristensen, H.L., 2016. Can living mulches in intercropping systems reduce the potential nitrate leaching? Studies of organic cauliflower (*Brassica oleracea* L. var. *botrytis*) and leek (*Allium porrum* L.) production across European conditions. Renew. Agric. Food Syst. FirstView, 1–16, http://dx.doi.org/10.1017/s1742170516000211.