



## Termination method and time of agro-ecological service crops influence soil mineral nitrogen, cabbage yield and root growth across five locations in Northern and Western Europe

Margita Hefner<sup>a</sup>, Stefano Canali<sup>b</sup>, Koen Willekens<sup>c</sup>, Peter Lootens<sup>c</sup>, Pauline Deltour<sup>d</sup>, Annelies Beeckman<sup>d</sup>, Donatienne Arlotti<sup>e</sup>, Kalvi Tamm<sup>f</sup>, Ingrid Bender<sup>g</sup>, Rodrigo Labouriau<sup>h</sup>, Hanne Lakkenborg Kristensen<sup>a,\*</sup>

<sup>a</sup> Department of Food Science, Aarhus University, 8200 Aarhus N, Denmark

<sup>b</sup> Consiglio per la Ricerca in Agricoltura e l'analisi dell'Economia Agraria, Centro di Ricerca Agricoltura e Ambiente (CREA-AA), 00184 Roma, Italy

<sup>c</sup> Plant Sciences Unit, Institute for Agricultural, Fisheries and Food Research, 9820 Merelbeke, Belgium

<sup>d</sup> INAGRO, 8800 Rumebeke-Beitem, Belgium

<sup>e</sup> Walloon Agricultural Research Centre, 5030 Gembloux, Belgium

<sup>f</sup> Department of Agrotechnology, Estonian Crop Research Institute, 48309 Jõgeva, Estonia

<sup>g</sup> Jõgeva Plant Breeding Department, Estonian Crop Research Institute, 48309 Jõgeva, Estonia

<sup>h</sup> Department of Mathematics, Aarhus University, 8000 Aarhus C, Denmark

### ARTICLE INFO

#### Keywords:

*Brassica oleracea*  
Roller-crimper  
Incorporation  
Cover crops  
Organic farming  
Winter pea  
Winter rye  
Winter barley

### ABSTRACT

Agro-ecological service crops (ASCs), also known as cover crops, green manures or catch crops, can improve organic vegetable production in terms of weed suppression, nitrogen (N) recycling, or addition of N through symbiotic N<sub>2</sub> fixation by legumes. Traditionally, ASCs are terminated through full incorporation into the soil (FI), but alternative termination without tillage is available by roller-crimping (RC). The applicability of RC to Northern and Western European climates has only been studied to a limited extent. The objective of this study was therefore to investigate the N dynamics involved in FI and RC of legume and cereal ASCs in organic cabbage production across Northern and Western European climates. Field experiments were conducted at one location in Estonia and in Denmark, and at three locations in Belgium during two cropping cycles (2015/2016 and 2016/2017) to assess the effect of ASC termination method in spring (FI and RC) and ASC species (pea, pea/cereal mixtures and cereals), compared with a bare soil control (BS), on soil mineral N content, N accumulation, yield, and root growth of a following crop of cabbage. Agro-ecological service crops, and in particular cereals, reduced N availability for succeeding cabbage compared with BS by 37–73%. Similar marketable cabbage yield was obtained under FI and BS in two out of four cases in Belgium, due to higher availability of soil mineral N when terminated earlier (April/May) than RC (May/June). However, FI reduced marketable yields by 36–98% in the other cases in Belgium and in two out of four cases in Estonia and Denmark, where FI was conducted in May/June. Overall, cabbage marketable yields were reduced by 68–100% under RC compared to BS and FI in seven out of nine cases mainly due to reduced soil mineral N availability. Soil mineral N availability was lower under RC likely because of a slower mineralisation of ASCs and soil organic matter. Besides, the termination of ASCs close to the time of cabbage planting under RC increased pre-emptive competition on N. Cabbage marketable yield could be maintained under RC at fertilisation by 236 kg N ha<sup>-1</sup> following pea in Denmark. Cabbage root growth was generally increased with higher above ground plant biomass across treatments in Estonia and Denmark, while the relationship was reverse under RC in East Flanders, indicating changes in resource allocation. Soil mineral N at harvest did not differ among treatments in deeper depth, showing no higher risk of N leaching from FI or RC. In conclusion, RC showed major yield reductions in the first and second year after conversion from spring tillage. Therefore, further investigations should verify improved yields under longer-term RC practice, e.g. due to build-up of soil organic matter, before implementation can be recommended in organic vegetable production in Northern and Western Europe.

**Abbreviations:** ASC, agro-ecological service crop; BS, bare soil; FI, full incorporation; N, nitrogen; N<sub>acc</sub>, nitrogen accumulation; RC, roller-crimping; Root, intensity<sub>mod</sub>, modified root intensity

\* Corresponding author.

E-mail address: [hanne.kristensen@food.au.dk](mailto:hanne.kristensen@food.au.dk) (H.L. Kristensen).

<https://doi.org/10.1016/j.eja.2020.126144>

Received 9 July 2019; Received in revised form 9 July 2020; Accepted 12 July 2020

Available online 12 August 2020

1161-0301/ © 2020 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license

(<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## 1. Introduction

The use of agro-ecological service crops (ASCs) is encouraged in organic farming as it provides several benefits to the environment and the agricultural system. The term ASC includes crops formerly known as cover crops, green manures or catch crops, which improve the agro-ecosystem not primarily in terms of higher yield, but in terms of enhanced ecosystem services (Canali et al., 2015), such as improved soil physical, chemical and biological properties, weed suppression (Blanco-Canqui et al., 2015) and reduction of nitrogen (N) leaching (Tonitto et al., 2006). Species of ASC crops differ in their ability to reduce soil mineral N, with cereals being more effective in soil N depletion than legumes (Tonitto et al., 2006; Tosti et al., 2014; Frasier et al., 2017). Legumes, however, add N to the system through biological N<sub>2</sub> fixation, and once incorporated increase the amount of N available for the succeeding crop (Ranells and Wagger, 1996; Gabriel and Quemada, 2011). To reduce N leaching and fertiliser use at the same time, cereals and legumes can be grown in a mixture (Sainju et al., 2005; Tosti et al., 2014). Despite the beneficial effects of ASCs, they can also result in pre-emptive competition, as they take up soil mineral N and other resources that would have otherwise been available for the succeeding crop, thereby reducing crop yields (Thorup-Kristensen, 1993).

Agro-ecological service crops can be terminated in a variety of ways: mowing and leaving ASCs at the soil surface, mowing and full incorporation (FI) of ASCs into the soil by tillage, or roller-crimping (RC) without soil tillage. A roller-crimper is a steel drum with metal slats arranged in a chevron pattern, which crushes and crimps the stems and leaves of ASCs without cutting them to prevent re-sprouting, which may occur after mowing (Ashford and Reeves, 2003). In this way, the rolled ASC forms a biomass mulching layer, which reduces weed emergence (Leavitt et al., 2011; Canali et al., 2013; Navarro-Miró et al., 2019a), thereby providing a tool to control weeds in organic no-tillage systems, where the use of herbicides is prohibited. Besides controlling weeds, RC reduced working hours and energy consumption (Canali et al., 2013; Navarro-Miró et al., 2019b).

Adequate termination of rye by the roller-crimper is achieved at flowering stage (anthesis) (Mirsky et al., 2009), which occurs in late May/early June under Northern and Western European climates. This implies, that ASC termination with the roller-crimper may happen later in the spring compared with FI, which can result in a greater effect of pre-emptive competition by ASCs (Thorup-Kristensen and Dresboll, 2010). Reduced N availability through pre-emptive competition might be challenging for vegetable production, of cabbage in particular, as cabbage is an N demanding crop that can take up approximately 300 kg ha<sup>-1</sup> N under organic cultivation (Thorup-Kristensen et al., 2012). Allowing for sufficient time between ASC termination and crop transplanting may compensate for the negative effect of pre-emptive competition, as the majority of N was released within 4 weeks after vetch incorporation (Poffenbarger et al., 2015).

Further, ASC decomposition and N release depend on the termination method: ASCs mineralise faster when incorporated into the soil than when left on the soil surface (Poffenbarger et al., 2015). Indeed, FI maintained and RC decreased yields compared with bare soil (BS): Endive and savoy cabbage yields following incorporated vetch ASC were comparable to fertilised BS in Italy (Campiglia et al., 2014), but vegetable yields were reduced under RC by 41–92% compared to BS in Minnesota, USA (Leavitt et al., 2011) and cabbage yield was reduced under RC by 26% compared with cut and removed ASC in NY, USA (Mochizuki et al., 2008). The termination method may affect N leaching risk in the following winter, whereby crop residues left on the surface under RC may increase the risk of N leaching due to the slower N release as was shown in a modelling study by Coppens et al. (2007). A recent Danish study showed ecosystem effects of RC (Hefner et al., 2020), which need to be verified across different agronomic conditions.

In Europe, RC has primarily been studied in a Mediterranean climate, where good results were obtained (Canali et al., 2013), but

knowledge regarding RC applicability in Northern and Western European climates is rare in vegetable production (Hefner et al., 2020). The objective of this study was to investigate the N dynamics involved in FI and RC of legume and cereal ASCs and their effect on organic cabbage yield and root growth across Northern and Western European climates, in countries such as Estonia, Denmark and Belgium. We hypothesised that (1) ASC species would differ in their ability to deplete soil mineral N, with cereals accumulating more N than legumes, (2) cabbage yield and root growth would be higher under BS, but similar between FI and RC, when terminated at the same time, but (3) cabbage yield and root growth would be reduced under RC due to stronger pre-emptive competition compared with FI and BS when termination was earlier under FI, and (4) the risk of N leaching would be greatest under RC due to delayed mineralisation from ASC material at the soil surface.

## 2. Material and methods

### 2.1. Field sites and experimental designs

The study was conducted at five locations in three countries. The geographical locations of the experimental sites were Jõgeva in Estonia (58°44'59.4" N and 26°24'54.0" E), Aarslev in Denmark (55°30'84.4" N and 10°43'93.9" E), Melle in East Flanders, Belgium (50°59'08.3" N and 3°47'12.3" E), Roeselare in West Flanders, Belgium (50°54'16.0" N and 3°07'39.5" E), and Boneffe in Wallonia, Belgium (50°60'98.5" N and 4°95'47.5" E). Fig. 1 shows the climate of the five locations during the experimental period. The soil types and properties of the five locations are listed in Table 1. Experiments were conducted during two cropping cycles (2015/2016 and 2016/2017), except in Wallonia, where the experiment failed in 2016 due to flooding. Monthly precipitation and average air temperature during the experimental period are shown in Fig. 1. Fields were managed according to European organic farming regulations in Estonia since 2009, in Denmark since 1996 (first year) and 2014 (second year), in East Flanders since 2010, in West Flanders since 2003, and in Wallonia since 1995. The experiment was conducted at the same site during two years in East Flanders, whereas experimental sites were relocated in the second year at the other locations.

The trials were conducted using a one factorial non-randomized complete block design in Estonia, a one factorial randomized complete block design in East Flanders, and a two factorial split-plot design on a randomized complete block in Denmark, West Flanders and Wallonia. The first factor was ASC species (Table 2) and the second factor was ASC termination (RC and FI) – this was the only factor in Estonia and East Flanders. Agro-ecological service crops were terminated at the same date under FI and RC in Estonia and Denmark, but were terminated 3–6 weeks earlier under FI than under RC in the three Belgian locations. Thus, the ASC termination method was coupled to termination time in the Belgian locations. Bare soil (BS) was included as the control at all locations. The field design included 3 blocks in Denmark and Estonia and four blocks in the three Belgian trials. White cabbage (*Brassica oleracea* L. convar. *capitata* (L.) Alef. var. *alba* DC) was grown as the main crop in all locations except in Wallonia, where red cabbage (*Brassica oleracea* L. convar. *capitata* (L.) Alef. var. *rubra* DC) was grown. Details of the field trials can be found in Table 2.

### 2.2. Agricultural management

The times of agricultural operations at the five locations are listed in Table 3. Soils were tilled prior to ASC sowing with a mouldboard plough in Estonia, Denmark and Wallonia, whereas non-inversion tillage with a chisel was conducted in East and West Flanders. The roller-crimper (Soldo, Italy), which was used in Estonia and Denmark, had a diameter of 0.6 m and a width of 2 m. The weight was adjusted to 800 kg in 2016 and 1200 kg in 2017 in Estonia and 930 kg in both years in Denmark by filling water into the hollow roller-crimper and by adding ballast (400 kg) to the roller-crimper in order to adjust to soil

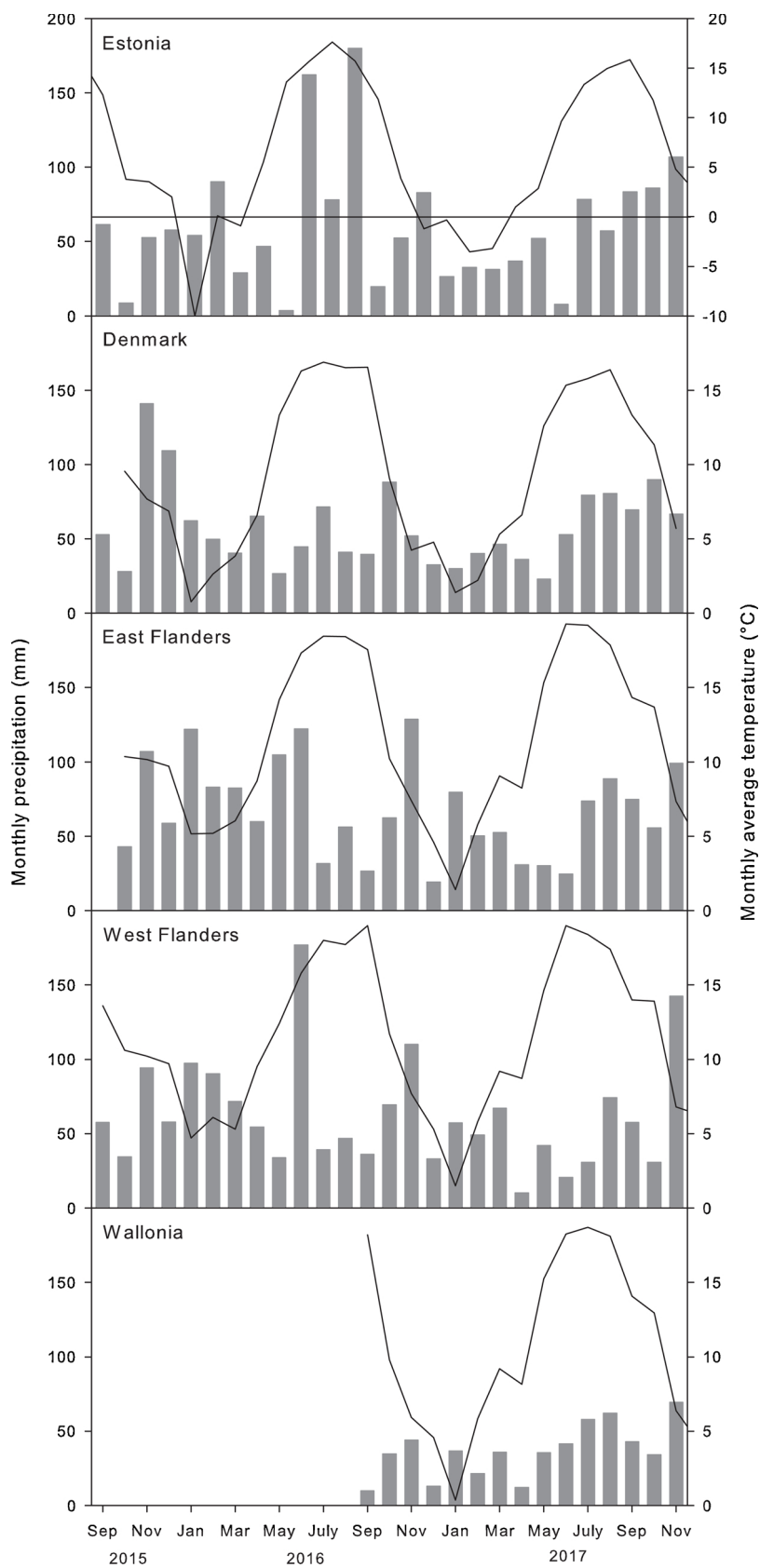


Fig. 1. Monthly average temperature (°C) and cumulative precipitation (mm) in all five locations from sowing of the ASC in 2015 until cabbage harvest in 2017.

**Table 1**  
Soil properties of experimental fields at five locations.

Soil depth (m)	Clay (%)	Silt (%)	Sand (%)	Soil depth (m)	C (%)	P (mg kg <sup>-1</sup> )	K (mg kg <sup>-1</sup> )	pH*
Estonia (sandy clay loam)								
0–0.35	29	17	54	0–0.3	2.5	93	106	6.9
0.35–0.40	28	17	55	0.3–0.6	1.5	53	56	–
0.40–0.75	8	4	89	0.6–0.9	1.9	19	54	–
0.75–1	29	14	58					
Denmark (sandy loam)								
0–0.5	13	15	70		1	23	115	6.7
0.5–1	18	13	68		0.2	19	98	5.9
1–2.5	18	14	68		0.1	16	105	7.3
East Flanders (sandy loam)								
0–0.3	14	20	66		1.1	270	151	5.7
0.3–0.6					0.5	95	110	5.7
West Flanders (sandy loam)								
0–0.3	31	9	60		1.1	400	215	6
0.3–0.6	28	12	60					
0.6–0.9	25	14	61					
Wallonia (silt loam)								
0–0.3 m	14	79	7		0.9	52	148	6.4

Note: P extracted with C<sub>3</sub>H<sub>9</sub>NO<sub>3</sub> in Estonia, East and West Flanders, with 0.5 M NaHCO<sub>3</sub> in Denmark, and with C<sub>3</sub>H<sub>9</sub>NO<sub>3</sub> and CH<sub>3</sub>COONH<sub>4</sub> in Wallonia. K extracted with CH<sub>3</sub>COONH<sub>4</sub> in Denmark, with C<sub>3</sub>H<sub>9</sub>NO<sub>3</sub> in Estonia, East and West Flanders, and with C<sub>3</sub>H<sub>9</sub>NO<sub>3</sub> and CH<sub>3</sub>COONH<sub>4</sub> in Wallonia. \* pH determination methods were 0.01 M CaCl<sub>2</sub> in Denmark, 1 M KCl in Estonia, East Flanders, West Flanders, and Wallonia.

moisture conditions at rolling. The weight was increased in the second year in Estonia in order to improve termination efficiency. The roller-crimper used in East and West Flanders was manufactured in East Flanders, whereas another roller-crimper (Renson, Belgium) was used in Wallonia. Both Belgian roller-crimpers had a diameter of 0.62 m, a width of 3 m, and a weight of 1720 kg. The weights of the roller-crimpers translate to 400 kg (2016), 600 kg (2017), 465 kg, and 573 kg per m in Estonia, Denmark and the Belgian locations, respectively. In Estonia and Denmark, roller-crimping was repeated after 1–2 weeks to improve termination efficiency. Agro-ecological service crops were roller-crimped with three passes in West Flanders. Cabbage was irrigated twice during the growing season in Denmark in 2016 and in West Flanders in 2016, once at planting in West Flanders in 2017, and before and after planting in East Flanders in 2017. Irrigation was not necessary at the other times and the other locations. Mechanical weeding was conducted in FI and BS plots in June and July and manual weeding in July, August and September in all locations. In addition, RC plots were weeded manually in Denmark in September 2016 and August 2017 and in Wallonia in July 2017. Cabbage plants were covered with nets to protect against insect pests or birds from planting to mid-August in Estonia, to mid-September in Denmark, to mid-July in East and West Flanders, and to mid-September in Wallonia. Further, cabbage plants were sprayed with *Bacillus thuringiensis* twice in Denmark in 2016, and one to three times per year in East Flanders.

### 2.3. Plant and soil sampling

Agro-ecological service crops were sampled at the respective termination dates given in Table 3. For ASC biomass determination an area of 1 m<sup>2</sup> was cut aboveground prior to ASC termination. In Wallonia an area of 2 m<sup>2</sup> was sampled. Cabbage yield at harvest was obtained by hand-harvesting the whole plot in Estonia, two rows × 4.5 m per plot in Denmark, eight rows × 0.7 m per plot in East Flanders, two rows × 2 m per plot in West Flanders, and three rows × 2.8 m per plot in Wallonia. Plant samples were divided into marketable yield, and crop residues and fresh weights were taken. Marketable yield was evaluated based on local standards by cabbage head size (minimum 400 g in Estonia, 500 g in Denmark, 12-cm diameter in East and West Flanders, and 13-cm diameter in Wallonia), and damage by pests or diseases according to the

local market standard. Plant material was chopped, mixed well, weighed, oven-dried to constant weight, weighed again and analysed by the combustion method for total plant N content. The measurement of ASC N content was missed in West Flanders (2016).

Soil mineral N and moisture content was determined at two to four times during the growing season, namely at ASC termination, 1–5 weeks after planting, at mid-season in August (6–12 weeks after planting) and at harvest (Table 3). In all Belgian locations, where ASC termination time between FI and RC differed, samples were taken at FI termination. Soil was sampled with a machine-driven soil piston auger with a 14-mm inner diameter in Denmark. In East Flanders, an Eijkelkamp auger with an inner diameter of 25 mm was used. A hand-driven auger was used in the other locations with 18-mm inner diameter in Estonia, and 13-mm inner diameter in West Flanders and Wallonia. Soil was sampled in 0–0.3 m, 0.3–0.6 m, 0.6–0.9 m soil depth layers in Estonia, East and West Flanders, in 0–0.25 m, 0.25–0.5 m, 0.5–1 m, 1–1.5 m, 1.5–2 m and 2–2.5 m soil depth layers in Denmark, and in 0–0.3 m soil depth in Wallonia. Four, ten, five, four or six, subsamples were taken per plot in Estonia, Denmark, East Flanders, West Flanders, and Wallonia, respectively, and mixed into one composite sample per soil layer. Soil mineral N was determined by extraction of nitrate and ammonium in either 2 M KCl (Estonia), 1 M KCl (Denmark, East and West Flanders) or 0.5 M KCl (Wallonia) for 1 h and analysis of the supernatant by standard colorimetric methods (ISO 14256–2). Soils were weighed, dried at 135 °C for 2 h in Estonia and at 105 °C for 24 h in Denmark and Wallonia, and weighed again to measure the moisture content.

### 2.4. Root measurement

Cabbage root growth was measured following rye in Estonia, following pea/rye in East Flanders and following pea in Denmark. In Estonia, root growth was assessed by taking 12 soil cores per plot (three soil cores around four cabbage plants), located 0.25 m away from the cabbage plants, with a hand-driven auger (18-mm inner diameter). Samples were taken from 0–0.3 m, 0.3–0.6 m, and 0.6–0.9 m depth on October 7th, 2016. In East Flanders, three soil cores per plot, located 5 cm away from the cabbage plants, were sampled with a hand-driven auger (80-mm inner diameter). The close sampling position was chosen

to ensure sufficient root biomass despite the poor crop development under RC. Samples were taken from 0–0.15 m and 0.3–0.45 m depth on August 9th, and November 7th, 2016, and August 22nd, and November 7th, 2017. Roots were extracted by rinsing with water on a sieve (1.20 mm in Estonia, 1 mm in East Flanders). In Estonia, roots were scanned with the scanner Perfection V700 Photo (Epson America Inc., USA) at an optical resolution of 400 dpi and images were analysed with the software WinRHIZO™ Pro 2017 (Regent Instruments Inc., Canada). In East Flanders, root dry weight was determined. In December 2019, additional white cabbage roots were sampled in East Flanders from another field and were scanned with the scanner Perfection V800 Photo (Epson America Inc., USA) at an optical resolution of 4800 dpi. Pictures were analysed with the macro IJ-Rhizo (Pierret et al., 2013) from the software ImageJ (National Institutes of Health, USA) and the resulting root length (derived from a corrected skeleton) was used to calculate a conversion factor per depth interval to transform root weight to root length.

In Denmark, root growth was measured in minirhizotrons, 3-m long transparent plastic tubes, which were installed shortly after cabbage transplanting. The minirhizotrons were prepared and inserted into the soil as described by Kristensen and Thorup-Kristensen (2004) at a 30° angle from vertical, reaching 2.4 m depth. Two tubes per plot were installed in the cabbage rows following winter pea. Roots were filmed with a mini-video camera two times during the crop growth on September 8th, and November 7th, 2016, and August 24th, and November 8th, 2017. Root intensity was obtained by counting the number of roots intersecting the two counting grids. Average root intensity was calculated per tube as the average sum of grid intersections within each 0.25-m soil layer. The root intensity was modified by modelling, as described in section 2.5, and was therefore called modified root intensity (root intensity<sub>mod</sub>)

## 2.5. Data analysis

ASC biomass was calculated as dry weight per area, whereas cabbage yield was calculated as fresh weight per area. Above ground N accumulation ( $N_{acc}$ ) was obtained from crop dry weight and N content. Soil mineral N was determined from the soil N concentration of the specific soil layers and the corresponding bulk density and subsequently summed for the observed soil profile. Bulk densities were measured in the experimental fields in Estonia (0–0.9 m), Denmark (0–0.25 m), West Flanders (0–0.3 m), and East Flanders (0–0.9 m). Bulk densities in 0.25–2.5 m depth were obtained from a previous experiment in the same field in Denmark, and reference values were used for 0.3–0.9 m depth in West Flanders and Wallonia. Root length density was calculated based on the root length obtained from the scanned images and the bulk density in Estonia. Root weight density was calculated as the root dry weight per soil volume and converted to root length density with the conversion factors obtained from the additional sampling in East Flanders.

Nitrogen balance was calculated as the difference between N input and output. The calculation was based on Kramberger et al. (2009) “ARNS” (apparent N remaining in the soil), but extended to include the autumn soil mineral N as follows:  $N \text{ balance (kg ha}^{-1}\text{)} = (N \text{ in above ground ASC biomass} + \text{soil mineral N at ASC termination} + N \text{ added with fertiliser}) - (N \text{ in above ground cabbage biomass} + \text{soil mineral N at harvest})$ . Soil mineral N at cabbage transplanting was used in Estonia and West Flanders (2017) instead of soil mineral N at ASC termination due to missing data. The soil mineral N content at ASC termination and at harvest was measured to varying soil depths (0–0.3 m depth in Wallonia, 0–0.9 m depth in Estonia, East and West Flanders and 0–2.5 m depth in Denmark). Potential N availability was estimated as the sum of ASC  $N_{acc}$ , spring soil mineral N in 0–0.9 m depth in all locations (excluding Wallonia), and fertiliser N. Spring soil mineral N was measured at ASC termination in Denmark and East Flanders and at cabbage transplanting in Estonia and West Flanders.

**Table 2**  
Details of planting and seeding of cabbage and ASC cultivars at five locations.

	Cabbage cultivar	ASC seeding rate (kg ha <sup>-1</sup> ) and cultivar	Plot size (m × m)	Row distance (m)	Plant distance (m)
Estonia	Jõgeva (2016, 2017)	180 Winter rye "Elvi" (2016, 2017)	6 × 4	0.5	0.5
Denmark	Coronet (2016, 2017)	192 Winter pea "James" (2016) 288 Winter pea "James" (2017) 96 Winter pea "Livado" (2016) 144 Winter pea "James" + 50 Winter rye "Livado" (2017)	3.2 × 10 (2016) 4.8 × 10 (2017)	0.5	0.5
East Flanders	Storema (2016) Kilozoc (2017)	75 Winter pea "Arkta" + 50 Winter rye "Palazzo" (2016) 100 Winter pea "Ascension" + 100 Winter rye "Matador" (2017)	7.5 × 15	0.7	0.35
West Flanders	Storema (2016) Kilozoc (2017)	100 Winter rye (2016, 2017) 120 Winter pea (2016) 70 Winter pea + 50 Winter rye (2016, 2017)	6 × 10, BS 6 × 9 (2016) 3 × 15 (2017)	0.7	0.3
Wallonia	Klimaro (2017)	120 Winter barley "Rafaela" (2017) 54 Winter pea "Arkta" + 59 Winter barley "Rafaela" (2017)	3 × 9	0.6	0.4

**Table 3**  
Times of agricultural operations at five locations from autumn 2015 to autumn 2017.

Field management	Estonia		Denmark		East Flanders		West Flanders		Wallonia	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
ASC sowing	Aug 25 2015	Sep 8 2016	Oct 5 2015	Oct 9 2016	Oct 5 2015	Nov 24 2016	Oct 13 2015	Oct 28 2016	Sep 15 2016	
FI termination										
- Mowed	June 6	June 16	June 10	May 30	Apr 20	May 7	Apr 29	Apr 18	May 5	
- Incorporated	June 6	June 16	June 10	May 30	May 4	June 7	May 9	Apr 20 & May 4	May 5	
RC termination	June 6	June 19	June 10	May 30	May 26	June 16	May 23	May 29	May 31	
Fertilisation (kg N ha <sup>-1</sup> )	168 cattle manure on July 28, 2015	153 cattle manure on Sep 9 2016, 12 horse manure compost on July 3	26 M13 on Sep 9 2015, 50 M13 on Aug 25	26 M13 on Sep 28 2016, 100 M13 & 30 lupine seeds on June 15, 80 M13 on Aug 24	90 farmyard manure on Oct 2 2015, 50 OPF on May 27	50 OPF on June 20	90 composted farmyard manure on Oct 12 2015, 50 OPF on July 8	50 OPF on Mar 16, 50 OPF on May 31, 75 OPF on July 25	82 cow manure on Aug 25 2016, 60 Terragr <sup>®</sup> on May 31	
Total fertilisation (kg N ha <sup>-1</sup> )	168	165	76	236	140	50	140	175	142	
Cabbage transplanting	June 13	June 19	July 1	June 21	May 27	June 20	June 23	May 30	May 31	
Harvest	Oct 7	Oct 4	Nov 15	Nov 2	Oct 26	Oct 30	Nov 21	Nov 7	Oct 25	

Note: M13 = Monterra 13 feather meal pellets (N:P-K: 13-0-0); OPF = Granular made of sugarcane extract (11-0-5); Terragr<sup>®</sup> = Granular made of slaughter house residues (9-5-0).

Statistical analyses were performed in R software, version 3.4.2 (R Core Team, 2017). Analyses of ASC biomass, cabbage yield, crop N<sub>acc</sub>, cabbage root growth and soil mineral N were conducted for locations and years separately. A generalized linear mixed model (GLMM) was defined by a Gaussian distribution containing two fixed effects (ASC species and termination method), and a random component representing the block and the whole-plot accounting for the split-plot design in Denmark, West Flanders and Wallonia. Bare soil was considered as an extra level under both factors. In Estonia and East Flanders termination method was the only fixed factor and the random component constituted of the block. In cases, where cabbage N<sub>acc</sub> and soil mineral N data did not follow a normal distribution with homogeneous variance, they were transformed by the function  $y = \log(x)$  or  $y = \sqrt{x}$ . The mixed models were defined with the R-package 'lme4', using likelihood based inference. Interactions between factors and main effects of factors were determined with an analysis of variance (ANOVA), followed by post-hoc analyses using the correction for multiple comparisons based on the method of False Discovery Rate (FDR) control (Benjamini and Yekutieli, 2001) with the R-package 'pairwise-Comparisons' (available at <http://home.math.au.dk/astatlab/software/pairwise-comparisons>). The effect of termination method on the regression coefficient (slope) of cabbage N<sub>acc</sub> versus potential N availability was determined with an ANOVA. Mean values are reported with standard errors and differences between treatments ( $P < 0.05$ ,  $P < 0.01$ , and  $P < 0.001$ ) are indicated by asterisks or different lowercase letters in figures. Root intensity<sub>mod</sub> was modelled with a GLMM defined by a Poisson distribution with a logarithmic link function, where termination method was the fixed factor and block and observations from the same minirhizotron tube were considered random components. The logarithm of the number of roots was set as an offset, which resulted in an arbitrary unit, explaining the lack of a unit. Details of the root intensity<sub>mod</sub> analysis method are described by Hefner et al., (2019). Root intensity<sub>mod</sub> figures report estimates of the model with 95% confidence intervals and not standard errors based on restrictions by the statistical method.

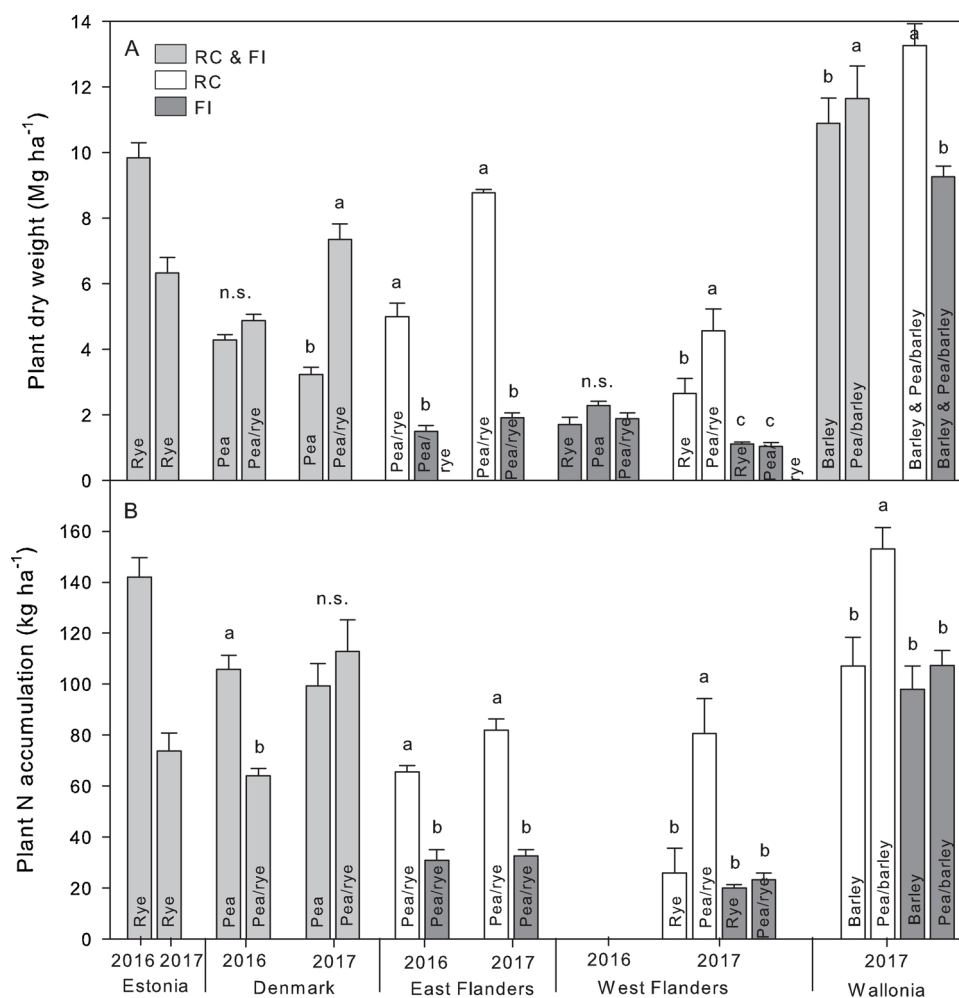
### 3. Results

#### 3.1. Plant yield and N accumulation

The earlier termination time of 3–6 weeks under FI in all Belgian locations resulted in increased ASC biomass and N<sub>acc</sub> under RC compared with FI, except for N<sub>acc</sub> of pure cereals (Fig. 2). Moreover, pea/cereal mixtures obtained more biomass than the pure crops in Denmark in both years (however, in 2016,  $P = 0.087$ ), under RC in West Flanders (2017), and in Wallonia (2017). The later termination time of ASCs under RC in the Belgian locations lead to an increased N<sub>acc</sub> of pea/cereal mixtures compared to pure cereals in West Flanders (2017), and in Wallonia (2017). Pea accumulated more N than pea/rye in Denmark in 2016, whereas no difference was observed in 2017.

In Estonia and Denmark, cabbage marketable yield and N<sub>acc</sub> were comparable under FI and BS in two out of four cases (Fig. 3). Marketable yield under FI was: five times higher than BS in Estonia (2016), at the same level as BS in Denmark (2017), but was reduced to 32% of BS in Estonia (2017), and 12% of BS in Denmark (2016) (Fig. 3 A). In Belgium, where FI was conducted earlier, marketable yield and N<sub>acc</sub> were comparable two and one out of four cases, respectively. Marketable yield under FI reached 92% of BS in East Flanders (2017), and 93% of BS in West Flanders (2017), but was reduced to 38% of BS in East Flanders (2016), and 43% of BS in Wallonia (2017).

In Estonia, marketable yield was four times higher under RC than FI in 2016, but was reduced to 2% of FI in 2017. Cabbage N<sub>acc</sub> followed a similar pattern. In Denmark, marketable yield was 0% of FI and of BS in 2016, but reached on average 77% of FI in 2017, where yield under RC pea was 97% of BS and under RC pea/rye was 60% of BS (Fig. 3 A). Cabbage N<sub>acc</sub> was maintained under RC compared with FI in Denmark



**Fig. 2.** Biomass (A) and N accumulation (B) of agro-ecological service crops at five locations. RC = roller-crimping, FI = full incorporation. Bars indicate standard error (Estonia and Denmark,  $n = 3$ ; East and West Flanders,  $n = 4$ ; and Wallonia, (A)  $n = 8$ , (B),  $n = 4$ ). N accumulation results are missing of West Flanders from 2016. Different lower-case letters indicate significant differences for location and year separately at  $P < 0.05$ ; n.s. = not significant.

in both years (Fig. 3 B). In Belgium, where RC was conducted later than FI, marketable yield and  $N_{acc}$  were reduced in all cases, reaching only 32% (2016) and 0% (2017) of FI in East Flanders, 19% (2016) and 0% (2017) of FI in West Flanders, and 4% of FI in Wallonia (2017).

Agro-ecological service crop species affected marketable yield and  $N_{acc}$  in three and two out of five cases, respectively: pea/rye reduced marketable yield and  $N_{acc}$  compared with pea under both RC and FI in Denmark in 2017, RC rye reduced marketable yield and  $N_{acc}$  compared with RC pea in West Flanders in 2016, and FI rye reduced marketable yield compared with BS in West Flanders in 2017.

### 3.2. Cabbage root growth

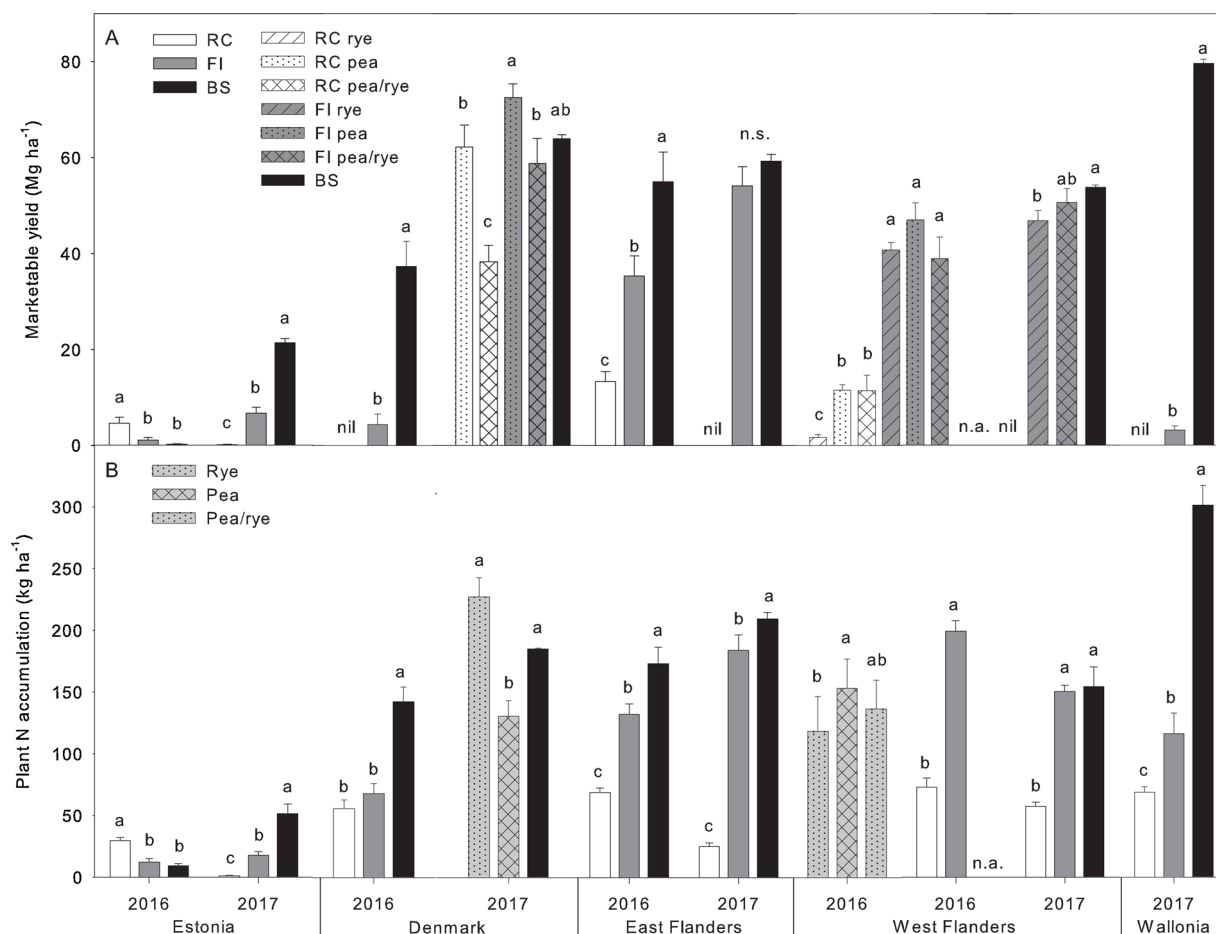
In Estonia, white cabbage root length density was higher under RC and FI compared with BS in 0–0.3 m depth and higher under FI than BS in 0.3–0.6 m depth at harvest in 2016 (Fig. 4 A). In Denmark, cabbage root intensity<sub>mod</sub> was similar between BS and FI in 0–0.75 m depth in August and November 2016, whereas it was lower under FI than BS below 0.75 m at both times (Fig. 5). In 2017, root intensity<sub>mod</sub> was lower under FI than BS only in 0–0.25 m depth in August and November. Root intensity<sub>mod</sub> was lower under RC than BS in the whole soil profile in August 2016, except in 0.75–1 m depth. In November 2016, root intensity<sub>mod</sub> was lower under RC than BS in 0–1.5 m depth, but differences were not significant in 0.75–1 m. No differences were observed between RC and BS in 2017. In East Flanders, cabbage root length density was the highest under RC in August 2016 and 2017

(0–0.15 m depth in August 2016 only by ANOVA) (Fig. 4 B–C). Root length density was the lowest in BS in August 2016, whereas it did not differ between BS and FI in August 2017. At harvest, root length density was higher under FI than in BS in 0–0.15 m in 2016, but did not differ among treatments in 0.3–0.45 m or in 2017 (Fig. 4 D–E).

### 3.3. Soil mineral N

ASC species affected soil mineral N content at three locations: Pea/rye reduced soil mineral N content compared with pea at ASC termination in Denmark in both years (Fig. 6 C and D). Rye reduced soil mineral N content compared with pea at ASC termination and at mid-season under FI in West Flanders in 2016 (Fig. 6 G), and barley reduced soil mineral N content compared with pea/barley under FI at ASC termination and at cabbage transplanting in Wallonia (Fig. 6 I). At mid-season, FI pea had the highest soil mineral N content compared with all other treatments in 0–0.3 m soil depth in Denmark in both years (supplementary material, Table S1).

Growing ASCs decreased soil mineral N content at ASC termination in the spring compared with BS by 85–157 kg ha<sup>-1</sup> in Denmark (0–2.5 m), 43–46 kg ha<sup>-1</sup> in East Flanders (0–0.9 m), 6 kg ha<sup>-1</sup> in West Flanders (0–0.9 m) (2016), and 21 kg ha<sup>-1</sup> in Wallonia (0–0.3 m) (Fig. 6). In Estonia, where termination of FI and RC was conducted at the same time, soil mineral N content was lower under FI and RC compared with BS at transplanting, but differences disappeared at mid-season and harvest (Fig. 6 A and B). In Belgium, however, where FI was



**Fig. 3.** Cabbage marketable yield (A) and N accumulation (B) at five locations. RC = roller-crimping, FI = full incorporation, BS = bare soil. Bars indicate standard error (Estonia,  $n = 3$ ; Denmark,  $n = 6$ , except yield 2017,  $n = 3$ ; East Flanders,  $n = 4$ ; West Flanders, (A)  $n = 4$ , (B),  $n = 8$  (species effect in 2016),  $n = 12$  (termination method effect in 2016),  $n = 8$  (2017); and Wallonia,  $n = 8$ ). Different lower-case letters indicate significant differences for location and year separately at  $P < 0.05$ ; n.s. = not significant; n.a. = not available; nil = zero yield.

conducted 3–6 weeks earlier than RC, soil mineral N content under FI was either intermediate between BS and RC (in East Flanders and Wallonia, Fig. 6 E, F and I), or it was similarly high as under BS and higher than under RC at transplanting (in West Flanders, Fig. 6 G and H). Similarly, soil mineral N content at mid-season was higher under BS and FI than under RC in East Flanders (2016 and 2017, Fig. 6 E and F) and in West Flanders (2016, Fig. 6 G). However, soil mineral N content did not differ among treatments at mid-season in West Flanders (2017, Fig. 6 H), and Wallonia (Fig. 6 I) and at harvest in most locations, except in East Flanders, where soil mineral N content was slightly higher under RC compared with FI and BS (2016 and 2017, Fig. 6 E and F). A closer look into the soil profile in 0–0.9 m depth revealed that differences were only prevalent in 0–0.3 m depth in East Flanders (Fig. 7 A and B). In West Flanders, soil mineral N content at harvest did not differ among treatments, except for a higher soil mineral N content under FI than RC in 0.3–0.6 m depth in 2017 (Fig. 7 C and D).

In 2016, soil moisture content was higher under RC and FI than BS in July and August in Estonia and in June in West Flanders. In 2017, soil moisture content was higher under RC than FI in August in Denmark, and it was the highest under RC, followed by FI and BS in June and August in West Flanders and Wallonia (supplementary material, Table S2).

### 3.4. Potential N availability, N accumulation, and N balance

The regression coefficients for RC ( $P < 0.05$ ), FI ( $P < 0.05$ ) and BS ( $P = 0.084$ ) (Fig. 8) show a positive relationship between potential

N availability and total cabbage  $N_{acc}$ . The coefficient under RC was by indication ( $P = 0.058$ ) higher than the one under BS. Differences between countries were evident, with the highest values for potential N availability and cabbage  $N_{acc}$  found in Denmark in 2017, followed by West Flanders, Denmark in 2016, East Flanders, and the lowest values in Estonia. This pattern reflects the different fertilisation strategies employed by the different locations, as potential N availability ranged from 33 to 417 kg N ha<sup>-1</sup>.

Nitrogen balance was the lowest in BS across all locations, except in Denmark in 2017 (Table 4). In Estonia and Denmark, where ASCs were terminated at the same time under RC and FI, N balance was similar under RC and FI, except for a lower N balance following FI pea/rye in 2016. In all Belgian locations, where ASCs were terminated 3–6 weeks earlier under FI than RC, N balance was the highest under RC, followed by FI, and BS. Differences between ASC species were evident under FI in Denmark in both years, where N balance was higher following pea than pea/rye.

## 4. Discussion

### 4.1. Spring soil mineral N affected by ASCs

Growing ASCs reduced soil mineral N in the spring (at ASC termination and at cabbage transplanting) compared to BS in all five locations studied here (Fig. 6), indicating a risk of pre-emptive competition by ASCs if N release is not synchronized with the subsequent crop's demand (Thorup-Kristensen, 1993). Agro-ecological service crop



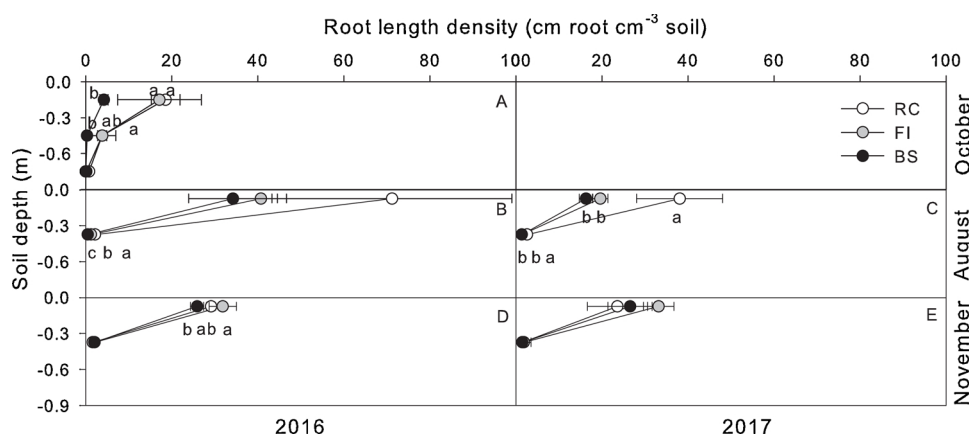


Fig. 4. Cabbage root length density in 0–0.9 m depth following rye in Estonia at harvest 2016 (A) and in 0–0.15 m and 0.3–0.45 m depth following pea/rye in East Flanders in August and November 2016 (B, D) and 2017 (C, E). RC = roller-crimping, FI = full incorporation, BS = bare soil. Bars indicate standard error (Estonia,  $n = 3$ ; East Flanders,  $n = 4$ ). Different letters indicate significant differences for each sampling time separately at  $P < 0.05$ .

species affected soil mineral N depletion in different ways, with cereals being more effective in depleting soil mineral N than pea, and cereal/pea mixtures showing intermediate effects (Fig. 6). Therefore, we support our first hypothesis that soil mineral N in spring was the lowest under pure cereals, followed by pea/cereal mixtures, pea and BS. Similar results were found in an Italian study, where nitrate concentration in drainage water at 0.9 m depth in the spring was lower under pure barley and barley/vetch than under vetch and BS (Tosti et al., 2014). This effect may be reinforced by ASCs' higher evapotranspiration lowering drainage volume compared to BS.

As a consequence of a higher N uptake, pea/rye biomass was higher than pure pea biomass in Denmark in 2017 and by indication in 2016 ( $P = 0.087$ ; Fig. 2 A). The more pronounced differences in 2017 could be linked to higher soil mineral N availability in spring in 2017 compared with 2016 (Fig. 6 C and D). Pea/cereal biomass and  $N_{acc}$  were higher than cereal biomass and  $N_{acc}$  in East Flanders under RC and in Wallonia (Fig. 2) due to symbiotic  $N_2$  fixation by pea in the mixture. In line with these findings, grass/legume mixtures obtained more biomass than either pure stand due to mutual stimulatory effects on N acquisition by grasses and symbiotic  $N_2$  fixation by legumes (Nyfeler et al., 2011). Interestingly, despite an indication for lower pea biomass in Denmark in 2016,  $N_{acc}$  of pea was higher than of pea/rye (Fig. 2), owing to a higher N concentration in plant tissues. In Italy, hairy vetch/oat obtained a higher biomass than pure hairy vetch, whereas  $N_{acc}$  was higher in pure hairy vetch than in the mixture (Campiglia et al., 2011), confirming the increased N concentration in legume tissues.

#### 4.2. Marketable cabbage yields after bare soil (control treatment)

Marketable cabbage yields under BS were in the range of expected organic yields of 40–70 Mg ha<sup>-1</sup> in East and West Flanders, Wallonia and Denmark in 2017 (unpublished results and personal communication, Guy Depraetere, Lieven Delanote, Alain Delvigne, Richard de Visser, advisors). However, marketable yields under BS were much lower in Estonia, although marketable yields of 30 Mg ha<sup>-1</sup> can be expected (unpublished results, Ingrid Bender). The low yields in 2016 can be ascribed to low precipitation in September 2016, when cabbage was not irrigated (Fig. 1). The low yields under BS in Denmark in 2016 can be ascribed to poor quality of seedlings and lack of nutrients during the growing season, indicated by lower soil mineral N at ASC termination in 2016 compared with 2017 (Fig. 6 C and D).

#### 4.3. Termination system effects on cabbage yield, root growth and N dynamics (Estonia and Denmark)

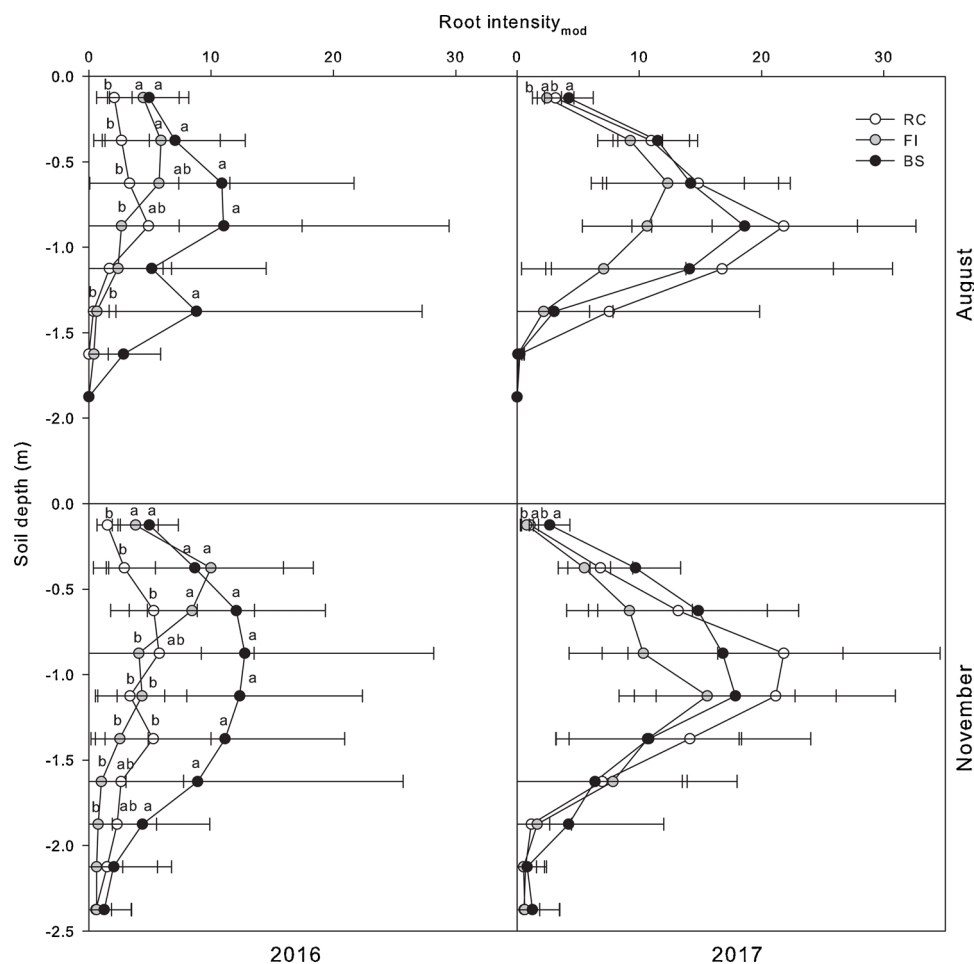
Both termination systems, RC and FI, reduced marketable cabbage yield and increased N balance compared with BS in Estonia in 2017 and in Denmark in 2016 (Fig. 3 and Table 4). The late ASC termination in June under RC and FI resulted in high  $N_{acc}$  by the ASC (64–142 kg N

ha<sup>-1</sup>) (Fig. 2 B) and a low soil mineral N content at termination in Denmark and at transplanting in Estonia (Fig. 6 A–D), both indicating a strong effect of pre-emptive competition. Similarly, delayed termination increased total N content of ASCs, but N release was offset by the chemical composition of the ASC (Wagger, 1989; Sainju and Singh, 2001). In agreement, pre-emptive competition was greater when ASCs were terminated later in the spring (Thorup-Kristensen and Dresboll, 2010; Alonso-Ayuso et al., 2014).

Considering the two termination systems, soil mineral N content throughout the growing season and N balance were comparable between RC and FI in Estonia and Denmark, except for a lower N balance following FI pea/rye in Denmark (Fig. 6 and Table 4). These similar conditions translated into comparable cabbage  $N_{acc}$  between termination systems in Denmark and even higher  $N_{acc}$  under RC in Estonia in 2016 (Fig. 2 B). However, RC reduced marketable yields by 98–100 % compared with FI in Estonia in 2017 and in Denmark in 2016, likely as the result of reduced N availability under RC. Nitrogen availability was lower under RC because of physical and biological protection of soil organic matter from mineralisation in soil aggregates (Beare et al., 1994; Kristensen et al., 2000), resulting in reduced N release from soil organic matter as compared to tilled soils under FI and BS. Moreover, N availability was limited under RC as a result of reduced potential N mineralisation and microbial activity as revealed by decreased dehydrogenase activity (Hefner et al., 2020). Nitrogen availability is further limited under RC because ASCs decompose and release N slower when left on the soil surface as compared to incorporation into the soil (Poffenbarger et al., 2015).

Comparable cabbage yield between BS and RC following pea in Denmark in 2017 (Fig. 3 A) can be ascribed to the combined effects of less pre-emptive competition and faster N release following pure pea, earlier fertiliser application, and increased fertilisation in 2017. Pre-emptive competition by pure pea was lower than by pea/rye as indicated by the higher soil mineral N content at ASC termination (Fig. 6 C and D). Net N release of pure pea was faster than of pea/rye, probably due to a lower C/N ratio (Hefner et al., 2020) and less lignified components, which was found for vetch compared with vetch/rye by Ranells and Wagger, (1996). Still, the positive yield response to RC pea was only observed in 2017, when 236 kg N ha<sup>-1</sup> fertiliser were added, whereas fertiliser addition was lower (50 kg N ha<sup>-1</sup>) and applied later in 2016 (Table 3), suggesting that sufficient N supply to cabbage is important in the early growth stage. Likewise, fertiliser timing affected broccoli yield in a German study, where low N supply at planting reduced yield, and at least 80–118 kg N ha<sup>-1</sup> and a second application 25 days after planting of 152–190 kg N ha<sup>-1</sup> was needed to obtain maximum yields (Feller and Fink, 2005). Furthermore, fertiliser was placed in the planting band under RC in Denmark in 2017, improving cabbage access to the fertiliser, whereas it was broadcast under FI and in BS.

Differences in cabbage root growth corresponded to differences in cabbage yield: both were the lowest in BS in Estonia in 2016 (Fig. 4 A),



**Fig. 5.** Cabbage root intensity<sub>mod</sub> following pea in 0–2.4 m depth in Denmark in August and November 2016 and 2017. RC = roller-crimping, FI = full incorporation, BS = bare soil. Bars indicate confidence intervals ( $n = 3$ ). Different letters indicate significant differences for each sampling time and depth separately at  $P < 0.05$ .

whereas they were the highest in BS, followed by FI and RC in Denmark in 2016 (Fig. 5). Root growth in 0.25–2.5 m depth did not differ between treatments in Denmark in 2017, when fertilisation was increased. However, cabbage yield was higher under FI pea than RC pea (Fig. 3), corresponding with higher soil mineral N content under FI pea in 0–0.3 m depth at mid-season (supplementary material, Table S1) This suggests that incorporated pea mineralised during this time and increased N availability for cabbage uptake, whereas this effect was smaller for pea/rye. This agrees with Hefner et al. (2020) and Poffenbarger et al. (2015), who found that potential N mineralisation and N release were greater from pure legumes compared with legumes/rye.

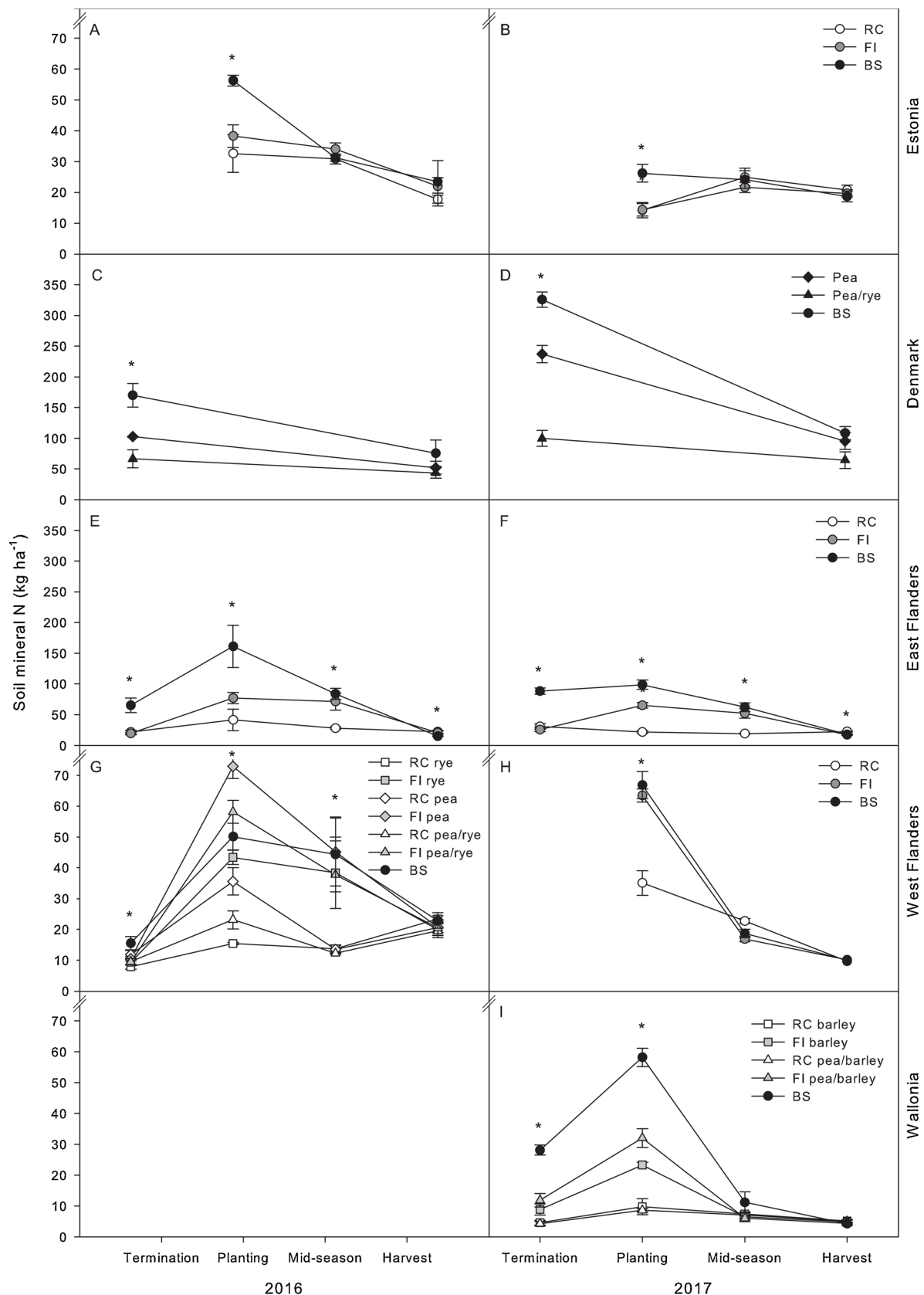
In conclusion, we partly support our second hypothesis that cabbage yield was higher in BS than under FI and RC in two out of four cases, because of reduced soil N availability following late ASC termination. Moreover, RC reduced cabbage yield compared with FI in three out of four cases, but maintained yield comparable to BS in Denmark in 2017 due to sufficient N supply from ASC peas and fertilisation.

#### 4.4. Termination system coupled with termination time effects on cabbage yield, root growth and N dynamics (Belgium)

The relatively early termination of ASCs under FI in late April or early May in all Belgian locations maintained cabbage yield comparable to BS in two out of four cases: in East Flanders in 2017 and following pea/cereals in West Flanders in 2017 (Fig. 3 A). Yields were maintained because pre-emptive competition was smaller with early ASC incorporation, as was shown by Thorup-Kristensen and Dresboll (2010).

Moreover, soil mineral N content at mid-season was similar between FI and BS in East Flanders in both years and at transplanting in West Flanders in 2017 (Fig. 6 E, F, H), resulting in comparable yields. Also, N balance was comparable between FI and BS in East Flanders in 2017 (Table 4), confirming that N dynamics were similar between treatments. In line with our findings, maize yield was not affected by replacing BS with barley or vetch ASCs in Spain (Gabriel and Quemada, 2011), indicating that FI did not restrict N supply compared with BS. However, FI reduced cabbage yield by 36% and 97%, and increased N balance in East Flanders in 2016 and in Wallonia in 2017. This shows that N supply from incorporated ASCs during the cabbage growing season did not always counterbalance the soil N depletion during ASC growth. Such pre-emptive competition may have been particularly high in Wallonia, where the water holding capacity of the silt loam soil was high, which showed reduced N leaching over winter and greater N availability for crop uptake. As a consequence, ASC biomass and  $N_{acc}$  were high under FI (Fig. 2).

In contrast to the generally moderate yield reductions under FI, RC reduced cabbage yield 71–100% compared with BS, and 61–100% compared with FI in all Belgian locations (Fig. 3 A). These yield reductions were higher than the 26% reduced cabbage yield under RC compared to cut and removed ASC in NY, USA (Mochizuki et al., 2008). Cabbage yield reductions corresponded to reduced cabbage  $N_{acc}$  (Fig. 3 B) and lower soil mineral N content under RC compared with BS and FI at cabbage transplanting in all Belgian locations (Fig. 6 E–I). In line with our findings, reduced soil mineral N content in 0–0.15 m depth contributed to vegetable yield reductions under RC compared with BS in Minnesota, USA (Leavitt et al., 2011). The reduced soil mineral N



**Fig. 6.** Soil mineral N content at five locations during the growing season in 2016 and 2017. RC = roller-crimping, FI = full incorporation, BS = bare soil. Note the change of scale on the y-axis due to sampling depths, which were 0–0.9 m in Estonia, East Flanders, and West Flanders, 0–2.5 m in Denmark, and 0–0.3 m in Wallonia. Sampled soil depth was 0–0.6 m in West Flanders at planting. Bars indicate standard error (Estonia, n = 3; Denmark, n = 3 at ASC period and n = 6 at harvest; East Flanders and Wallonia, n = 4; West Flanders, n = 4 (2016) and n = 8 (2017)). \* indicates significant differences for location, year, and sampling time separately at  $P < 0.05$ .

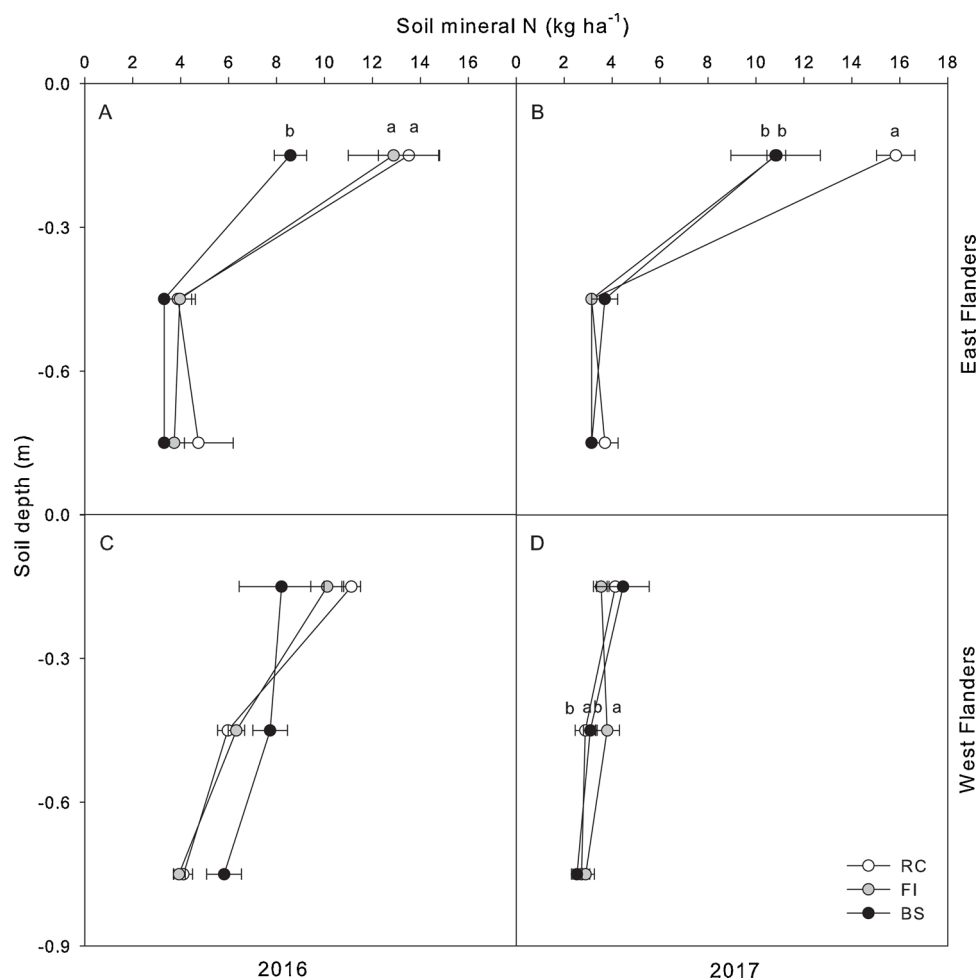


Fig. 7. Soil mineral N in 0–0.9 m depth in East and West Flanders at harvest in November 2016 and 2017. RC = roller-crimping, FI = full incorporation, BS = bare soil. Bars indicate standard error (East Flanders,  $n = 4$ ; West Flanders,  $n = 8$ ). Lower case letters indicate significant differences for location, year, and sampling depth separately at  $P < 0.05$ .

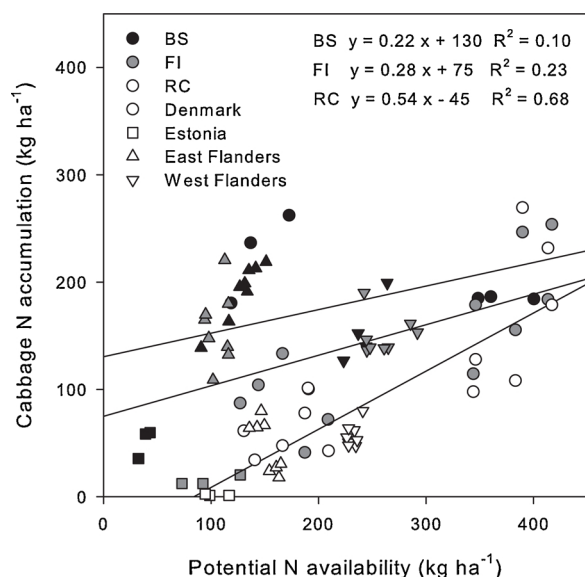
content was likely due to the combined effects of later termination and absence of incorporation under RC compared with FI. The later ASC termination under RC resulted in a greater soil mineral N uptake during their growth, evident by the higher  $N_{acc}$  (Fig. 2 B), and therefore in a greater effect of pre-emptive competition compared to FI. Furthermore, C/N ratio of ASC biomass shifts towards higher values for plants at a later growth stage, and mineralisation was thereby reduced after ASC termination (Thorup-Kristensen and Dresboll, 2010), although ASC  $N_{acc}$  was higher under RC (Fig. 2 B). Similarly, ASCs terminated late had a higher fiber content and C/N ratio, which made it more resistant to decomposition, thereby slowing down the N release (Alonso-Ayuso et al., 2014). As already discussed in section 4.3, N availability was also reduced under RC due to protection of soil organic matter and slower N release from surface-placed ASC biomass (Beare et al., 1994; Poffenbarger et al., 2015).

Cabbage  $N_{acc}$  was the lowest under RC in most cases, even if potential N availability was similar among RC, FI and BS (Fig. 8). Although partly explained by the incomplete release of the organically bound N, this suggests that other factors besides N availability affected cabbage yield under RC. Despite evidence that ASC residues conserve soil moisture (Teasdale and Mohler, 1993; Canali et al., 2013), soil moisture content in West Flanders and Wallonia in June and August 2017 were the lowest under RC, followed by FI and BS (supplementary material, Table S2), which might have affected yield negatively. Possible explanations for reduced soil moisture under RC include the delayed termination compared with FI, which implies that ASCs

continued to use water during the longer growing phase under RC, causing pre-emptive competition for water. Moreover, water use by ASCs may have continued during the slower decomposition following RC in contrast to FI. These findings are in line with the increased pre-emptive competition of water with delayed termination date found in Spain (Alonso-Ayuso et al., 2014). Other factors causing yield reductions under RC could be compacted soil in 0–0.25 m depth as a result of no-tillage (Bulan et al., 2015) at ASC termination, reduced soil temperature due to ASC biomass protecting the soil surface from heating, and inconsistent weed suppression later in the growing season (Hefner et al., 2020).

The higher regression coefficient of cabbage  $N_{acc}$  related to potential N availability under RC compared with BS (Fig. 8) suggests that cabbage was more responsive and dependent on N availability under RC. Potential N availability levels were most probably not achieved during the early growing phase to meet crop N demand under RC conditions, due to limited release of N from soil organic matter and slow mineralisation of ASC material on the soil surface. High levels of N were left under RC, as indicated by the higher N balance values under RC than under FI and/or BS in all Belgian locations (Table 4). This strongly indicates a poorer synchronisation of N availability with crop N demand under RC systems and the need for sufficient fertilisation as was the case in the Danish pea treatment in 2017.

Interestingly, cabbage root growth was higher under RC than FI and BS in East Flanders in August in both years, but differences disappeared at harvest, probably due to very dense rooting in the top soil (Fig. 4



**Fig. 8.** Total cabbage N accumulation at harvest related to potential N availability (fertiliser N + soil mineral N in 0–0.9 m depth in spring + N accumulated by agro-ecological service crops). Every data point represents the relation between N accumulation and potential N availability in each plot and location each year, with the exception of three cases: Estonia 2016 due to low yields, West Flanders 2016 due to absence of ASC  $N_{acc}$  data, and Wallonia due to limited soil depth of 0–0.3 m. RC = roller-crimping, FI = full incorporation, BS = bare soil. Lines show the linear regression for RC, FI and BS.

B–E). Remaining roots from ASCs under RC could have increased the measured root biomass in August, however, [Sievers and Cook \(2018\)](#) found that only approximately 8% and 20% of vetch and cereal root biomass, respectively, remained in the soil 10 weeks after ASC termination, which corresponded to the time between ASC termination and root sampling in our study. The higher root density but lower above ground biomass of cabbage under RC indicate a change in resource allocation between shoot and root, which might have been caused by limited N availability under RC. In accordance, oilseed rape (*Brassica napus* L.) root production was greater but biomass was reduced without fertiliser application compared with fertiliser application ([Kamh et al., 2005](#)).

We confirm our third hypothesis that cabbage yield was reduced under RC compared with FI and BS when termination was later under RC. Yields were reduced under RC because of greater pre-emptive competition, slower N mineralisation of ASCs and soil organic matter, and reduced soil moisture. Temperature and increased weed growth

later in the growing season may have contributed as well. However, root growth was increased under RC, whereas above ground biomass was decreased in East Flanders, indicating a change in resource allocation.

#### 4.5. Nitrogen leaching risk in autumn under RC

Soil mineral N content in autumn did not differ among treatments in all locations, except in Denmark (2017) and East Flanders (2016 and 2017), where soil mineral N was higher under RC than FI and BS, indicating that N availability was not coupled with cabbage N demand under RC. Plant material of ASCs increased the mineral N pool under RC in the top soil, possibly providing a source for increased N leaching over winter. Similarly, larger net N accumulation under ASC mulch compared to FI potentially increased the risk of nitrate leaching if no ASCs would be planted in the following autumn ([Coppens et al., 2007](#); [Campiglia et al., 2011](#)). However, differences among treatments were small ([Fig. 6](#)) and only occurred in 0–0.3 m depth ([Fig. 7](#)). Further, soil mineral N content in autumn did not differ among treatments in six out of nine cases, suggesting that N leaching risk was not increased under RC and therefore we reject our fourth hypothesis. In addition, ASCs were indicated to decrease leaching the preceding winter compared to BS, as soil mineral N content was reduced under RC and FI compared with BS in most locations at ASC termination and cabbage transplanting ([Fig. 6](#)). Therefore, ASCs acted as N catch crops ([Tonitto et al., 2006](#)).

#### 4.6. Assessment of termination system and time

The difference in termination time of FI, with earlier termination respective to RC in all Belgian locations but same termination time as RC in Estonia and Denmark, resulted in generally greater differences between termination systems in all Belgian locations. In order to assess the sole effect of termination method, results from RC and FI conducted at the same time need to be considered, as was the case in Estonia and Denmark. Here, differences in soil mineral N at transplanting and N balance between termination systems were small. Still, cabbage yield was only maintained in two out of four cases under RC compared with BS (in Estonia in 2016 and in Denmark in 2017). Several factors contributed to acceptable cabbage yields under RC in Denmark in 2017: N availability was increased because pea was chosen as an ASC, cabbage was fertilised at planting, fertiliser was placed in a planting-band and fertilisation was increased compared with 2016.

Nonetheless, RC reduced cabbage yields in seven out of eight cases, partly as the result of greater pre-emptive competition. The importance of adequate time for mineralisation of ASC biomass is well known after ASC termination before planting a next crop ([Thorup-Kristensen and Dresboll, 2010](#)). This timing may have been violated by the need to

**Table 4**

Nitrogen balance ( $\text{kg N ha}^{-1}$ ) between termination of agro-ecological service crops in spring and cabbage harvest in autumn at five locations.

Termination method	ASC species	Estonia		Denmark		East Flanders		West Flanders	Wallonia
		2016	2017	2016	2017	2016	2017	2017	2017
RC	Pea	120 ± 10 <sup>a</sup>	81 ± 6 <sup>a</sup>	130 ± 28 <sup>a</sup>	211 ± 24 <sup>ab</sup>	47 ± 5 <sup>a</sup>	118 ± 4 <sup>a</sup>	143 ± 3 <sup>a</sup>	121 ± 10 <sup>a</sup>
	Pea/rye			137 ± 25 <sup>a</sup>	247 ± 38 <sup>ab</sup>				
FI	Pea	148 ± 13 <sup>a</sup>	63 ± 11 <sup>a</sup>	98 ± 9 <sup>a</sup>	263 ± 28 <sup>a</sup>	−52 ± 8 <sup>b</sup>	−89 ± 9 <sup>b</sup>	108 ± 5 <sup>b</sup>	34 ± 4 <sup>b</sup>
	Pea/rye			19 ± 15 <sup>b</sup>	192 ± 19 <sup>b</sup>				
BS		24 ± 5 <sup>b</sup>	−32 ± 6 <sup>b</sup>	−82 ± 18 <sup>c</sup>	242 ± 6 <sup>ab</sup>	−78 ± 4 <sup>c</sup>	−82 ± 1 <sup>b</sup>	77 ± 10 <sup>c</sup>	−217 ± 15 <sup>c</sup>

Note: ASC = agro-ecological service crop, RC = roller-crimping, FI = full incorporation, BS = bare soil. N balance = (N in above ground ASC biomass + soil mineral N at ASC termination + N added with fertiliser) − (N in above ground cabbage biomass + soil mineral N at harvest). Soil mineral N was measured in 0–0.9 m depth in Estonia, East Flanders and West Flanders, in 0–2.5 m depth in Denmark and in 0–0.3 m depth in Wallonia. In Estonia and West Flanders, soil mineral N at cabbage transplanting was used instead of at ASC termination. Mean values are followed by standard error (Estonia and Denmark, n = 3; East and West Flanders, n = 4; Wallonia, n = 8). Results are missing in West Flanders from 2016 due to absence of ASC  $N_{acc}$  data. Different superscript letters indicate significant differences among treatments at  $P < 0.01$ .

reach anthesis/flowering of the ASC for effective RC termination (Mirsky et al., 2009). This implies that adapting ASC cycles with faster crop development, e.g. by choosing different ASC species, are needed for RC systems in order to enable earlier termination, thereby improving N availability and potentially yield in this system.

This study reflects changes in the early conversion phase (one to two seasons) from a tilled to a reduced tilled or no-tillage system. After 7–9 years of no-tillage implementation, total soil organic N, potentially available N and total soil carbon were accumulated in 0–0.05 m depth under no-tillage, whereas organic matter distribution was more uniform under conventional tillage (Haynes and Knight, 1989). The accumulated organic matter at the soil surface under RC together with the absence of soil disturbance by tillage might in the medium and long-term result in a build-up of soil organic matter and increased N availability if implemented over several years.

## 5. Conclusion

Agro-ecological service crops depleted soil mineral N during their growth, resulting in reduced N availability for succeeding cabbage compared with BS in all five locations considered in this study. This reduction was greater by cereal ASCs compared with legume ASCs, due to less soil mineral N depletion by pea, which accumulated N through symbiotic N<sub>2</sub> fixation. Cabbage yield was maintained under FI compared with BS in two out of four cases in Belgium, probably due to limited pre-emptive competition when ASCs were terminated early in the growing season (April/May). Still, yield reductions following FI were observed in some years and locations possibly due to seasonal and local factors increasing pre-emptive competition and reducing N release. Roller-crimping reduced cabbage yield in the short-term (one to two seasons) in seven out of nine cases, which can partly be explained by reduced soil mineral N availability. Soil mineral N content was reduced under RC because of slower mineralisation from ASCs at the soil surface and untilled soil. However, cabbage yield and root growth were equally high under RC pea compared with BS in Denmark in 2017, owing to higher fertiliser input and N availability from mineralised pea, suggesting that RC could work at improved fertiliser management. Soil mineral N content was higher under RC at harvest in the top soil in three cases, but did not differ among treatments in deeper depth, indicating no increased risk of N leaching from FI or RC. Root growth response was variable, correlating to above ground plant biomass in some cases (Estonia and Denmark), while higher root density at lower above ground biomass was found under RC in East Flanders, indicating changes in resource allocation. Before RC can be recommended for organic vegetable production in Northern and Western European climates, further research needs to focus on the possibility to employ ASC species with faster growth cycles, improved fertilisation management and possible beneficial long-term effects on a build-up of soil organic matter.

## Data statement

Data sets are not specified but can be made available upon request.

## CRediT authorship contribution statement

**Margita Hefner:** Formal analysis, Investigation, Writing - original draft, Visualization. **Stefano Canali:** Conceptualization, Methodology, Project administration, Funding acquisition, Writing - review & editing. **Koen Willekens:** Funding acquisition, Investigation, Writing - review & editing. **Peter Lootens:** Investigation, Writing - review & editing. **Pauline Deltour:** Investigation, Writing - review & editing. **Annelies Beeckman:** Investigation. **Donatienne Arlotti:** Investigation, Writing - review & editing. **Kalvi Tamm:** Investigation, Writing - review & editing. **Ingrid Bender:** Funding acquisition, Investigation, Writing - review & editing. **Rodrigo Labouriau:** Methodology, Formal analysis.

**Hanne Lakkenborg Kristensen:** Funding acquisition, Methodology, Investigation, Supervision, Writing - review & editing.

## Declaration of Competing Interest

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

## Acknowledgement

This study was part of the SoilVeg research project and was funded by ERA-NET CORE Organic PLUS and several national funding bodies. We thank Ülle Ratassepp, Taavi Piiskoppel, Larissa Sirel, Siiri Margens, Astrid Bergmann, Lasse Vesterholt, Knud Erik Pedersen, Jens Barford, Jens Elkjær, Bjarne Egedal, the technical staff at the Institute for Agricultural, Fisheries and Food Research in East Flanders, Brecht Vandebroucke, Tom De Cuypere, Lieven Delanote, Véronique Reuter, Frédérique Tasiaux, Joël Frédérick, Véronique Vrancken, Daniel Charles, Eloise Ruth, and Johan Verwijvel for carrying out the field experiments.

## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.eja.2020.126144>.

## References

- Alonso-Ayuso, M., Luis Gabriel, J., Quemada, M., 2014. The kill date as a management tool for cover cropping success. *PLoS One* 9. <https://doi.org/10.1371/journal.pone.0109587>.
- Ashford, D.L., Reeves, D.W., 2003. Use of a mechanical roller-crimper as an alternative kill method for cover crops. *Am. J. Alternative Agric.* 18, 37–45. <https://doi.org/10.1079/AJAA200232>.
- Beare, M.H., Cabrera, M.L., Hendrix, P.F., Coleman, D.C., 1994. Aggregate-protected and unprotected organic matter pools in conventional tillage and no-tillage soils. *Soil Sci. Soc. Am. J.* 58, 787–795. <https://doi.org/10.2136/sssaj1994.03615995005800030021x>.
- Benjamini, Y., Yekutieli, D., 2001. The control of the false discovery rate in multiple testing under dependency. *Ann. Stat.* 29, 1165–1188.
- Blanco-Canqui, H., Shaver, T.M., Lindquist, J.L., Shapiro, C.A., Elmore, R.W., Francis, C.A., Hergert, G.W., 2015. Cover crops and ecosystem services: insights from studies in temperate soils. *Agron. J.* 107, 2449–2474. <https://doi.org/10.2134/agronj15.0086>.
- Bulan, M.T.S., Stoltenberg, D.E., Posner, J.L., 2015. Buckwheat species as summer cover crops for weed suppression in no-tillage vegetable cropping systems. *Weed Sci.* 63, 690–702. <https://doi.org/10.1614/ws-d-14-00088.1>.
- Campiglia, E., Mancinelli, R., Radicetti, E., 2011. Influence of no-tillage and organic mulching on tomato (*Solanum lycopersicum* L.) production and nitrogen use in the Mediterranean environment of central Italy. *Sci. Hortic.* 130, 588–598. <https://doi.org/10.1016/j.scienta.2011.08.012>.
- Campiglia, E., Mancinelli, R., Di Felice, V., Radicetti, E., 2014. Long-term residual effects of the management of cover crop biomass on soil nitrogen and yield of endive (*Cichorium endivia* L.) and savoy cabbage (*Brassica oleracea* var. *sabauda*). *Soil Till. Res.* 139, 1–7. <https://doi.org/10.1016/j.still.2014.01.003>.
- Canali, S., Campanelli, G., Ciaccia, C., Leteo, F., Testani, E., Montemurro, F., 2013. Conservation tillage strategy based on the roller crimper technology for weed control in Mediterranean vegetable organic cropping systems. *Eur. J. Agron.* 50, 11–18. <https://doi.org/10.1016/j.eja.2013.05.001>.
- Canali, S., Diacono, M., Campanelli, G., Montemurro, F., 2015. Organic no-till with roller crimpers: agro-ecosystem services and applications in organic Mediterranean vegetable productions. *Sustain. Agric. Res.* 4, 70–79.
- Coppens, F., Garnier, P., Findeling, A., Merckx, R., Recous, S., 2007. Decomposition of mulched versus incorporated crop residues: modelling with PASTIS clarifies interactions between residue quality and location. *Soil Biol. Biochem.* 39, 2339–2350. <https://doi.org/10.1016/j.soilbio.2007.04.005>.
- Feller, C., Fink, M., 2005. Growth and yield of broccoli as affected by the nitrogen content of transplants and the timing of nitrogen fertilization. *HortScience* 40, 1320–1323.
- Frasier, I., Noellemeier, E., Amiotti, N., Quiroga, A., 2017. Vetch-rye biculture is a sustainable alternative for enhanced nitrogen availability and low leaching losses in a no-till cover crop system. *Field Crops Res.* 214, 104–112. <https://doi.org/10.1016/j.fcr.2017.08.016>.
- Gabriel, J.L., Quemada, M., 2011. Replacing bare fallow with cover crops in a maize cropping system: yield, N uptake and fertiliser fate. *Eur. J. Agron.* 34, 133–143. <https://doi.org/10.1016/j.eja.2010.11.006>.

- Haynes, R.J., Knight, T.L., 1989. Comparison of soil chemical properties, enzyme activities, levels of biomass N and aggregate stability in the soil profile under conventional and no-tillage in Canterbury, New Zealand. *Soil Till. Res.* 14, 197–208. [https://doi.org/10.1016/0167-1987\(89\)90008-1](https://doi.org/10.1016/0167-1987(89)90008-1).
- Hefner, M., Labouriau, R., Nørremark, M., Kristensen, H.L., 2019. Controlled traffic farming increased crop yield, root growth, and nitrogen supply at two organic vegetable farms. *Soil Till. Res.* 191, 117–130. <https://doi.org/10.1016/j.still.2019.03.011>.
- Hefner, M., Gebremikael, M.T., Canali, S., Sans Serra, F.X., Petersen, K.K., Sorensen, J.N., De Neve, S., Labouriau, R., Kristensen, H.L., 2020. Cover crop composition mediates the constraints and benefits of roller-crimping and incorporation in organic white cabbage production. *Agric. Ecosyst. Environ.* 296, 106908. <https://doi.org/10.1016/j.agee.2020.106908>.
- Kamh, M., Wiesler, F., Ulas, A., Horst, W.J., 2005. Root growth and N-uptake activity of oilseed rape (*Brassica napus* L.) cultivars differing in nitrogen efficiency. *J. Plant Nutr. Soil Sci.* 168, 130–137. <https://doi.org/10.1002/jpln.200421453>.
- Kramberger, B., Gselman, A., Janzekovic, M., Kaligarić, M., Bracko, B., 2009. Effects of cover crops on soil mineral nitrogen and on the yield and nitrogen content of maize. *Eur. J. Agron.* 31, 103–109. <https://doi.org/10.1016/j.eja.2009.05.006>.
- 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., McCarty, G.W., Meisinger, J.J., 2000. Effects of soil structure disturbance on mineralization of organic soil nitrogen. *Soil Sci. Soc. Am. J.* 64, 371–378. <https://doi.org/10.2136/sssaj2000.641371x>.
- Leavitt, M.J., Sheaffer, C.C., Wyse, D.L., Allan, D.L., 2011. Rolled winter rye and hairy vetch cover crops lower weed density but reduce vegetable yields in no-tillage organic production. *HortScience* 46, 387–395.
- Mirsky, S.B., Curran, W.S., Mortensen, D.A., Ryan, M.R., Shumway, D.L., 2009. Control of cereal rye with a roller/crimper as influenced by cover crop phenology. *Agron. J.* 101, 1589–1596. <https://doi.org/10.2134/agronj2009.0130>.
- Mochizuki, M.J., Rangarajan, A., Bellinder, R.R., van Es, H.M., Bjorkman, T., 2008. Rye mulch management affects short-term indicators of soil quality in the transition to conservation tillage for cabbage. *HortScience* 43, 862–867.
- Navarro-Miró, D., Blanco-Moreno, J.M., Ciaccia, C., Chamorro, L., Testani, E., Kristensen, H.L., Hefner, M., Tamm, K., Bender, I., Jakop, M., Bavec, M., Védie, H., Lepse, L., Canali, S., Sans, F.X., 2019a. Agroecological service crops managed with roller crimper reduce weed density and weed species richness in organic vegetable systems across Europe. *Agron. Sust. Dev.* 39. <https://doi.org/10.1007/s13593-019-0597-8>.
- Navarro-Miró, D., Iocola, I., Persiani, A., Blanco-Moreno, J.M., Kristensen, H.L., Hefner, M., Tamm, K., Bender, I., Védie, H., Willekens, K., Diacono, M., Montemurro, F., Sans, F.X., Canali, S., 2019b. Energy flows in European organic vegetable systems: effects of the introduction and management of agroecological service crops. *Energy* 188, 116096. <https://doi.org/10.1016/j.energy.2019.116096>.
- Nyfelner, D., Huguenin-Elie, O., Matthias, S., Frossard, E., Luscher, A., 2011. Grass-legume mixtures can yield more nitrogen than legume pure stands due to mutual stimulation of nitrogen uptake from symbiotic and non-symbiotic sources. *Agric. Ecosyst. Environ.* 140, 155–163. <https://doi.org/10.1016/j.agee.2010.11.022>.
- Pierret, A., Gonkhamdee, S., Jourdan, C., Maeght, J.L., 2013. IJ\_Rhizo: an open-source software to measure scanned images of root samples. *Plant Soil* 373, 531–539. <https://doi.org/10.1007/s11104-013-1795-1799>.
- Poffenbarger, H.J., Mirsky, S.B., Weil, R.R., Kramer, M., Spargo, J.T., Cavigelli, M.A., 2015. Legume proportion, poultry litter, and tillage effects on cover crop decomposition. *Agron. J.* 107, 2083–2096. <https://doi.org/10.2134/agronj15.0065>.
- R Core Team, 2017. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.
- Ranells, N.N., Wagger, M.G., 1996. Nitrogen release from grass and legume cover crop monocultures and bicultures. *Agron. J.* 88, 777–782. <https://doi.org/10.2134/agronj1996.00021962008800050015x>.
- Sainju, U.M., Singh, B.P., 2001. Tillage, cover crop, and kill-planting date effects on corn yield and soil nitrogen. *Agron. J.* 93, 878–886. <https://doi.org/10.2134/agronj2001.934878x>.
- Sainju, U.M., Whitehead, W.F., Singh, B.P., 2005. Biculture legume-cereal cover crops for enhanced biomass yield and carbon and nitrogen. *Agron. J.* 97, 1403–1412. <https://doi.org/10.2134/agronj2004.0274>.
- Sievers, T., Cook, R.L., 2018. Aboveground and root decomposition of cereal rye and hairy vetch cover crops. *Soil Sci. Soc. Am. J.* 82, 147–155. <https://doi.org/10.2136/sssaj2017.05.0139>.
- Teasdale, J.R., Mohler, C.L., 1993. Light transmittance, soil-temperature, and soil-moisture under residue of hairy vetch and rye. *Agron. J.* 85, 673–680.
- Thorup-Kristensen, K., 1993. The effect of nitrogen catch crop species on the nitrogen nutrition of a succeeding crop: I. Effects through mineralization and pre-emptive competition. *Acta Agric. Scand. B* 43, 74–81. <https://doi.org/10.1080/09064719309411222>.
- Thorup-Kristensen, K., Dresboll, D.B., 2010. Incorporation time of nitrogen catch crops influences the N effect for the succeeding crop. *Soil Use Manage.* 26, 27–35. <https://doi.org/10.1111/j.1475-2743.2009.00255.x>.
- 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. <https://doi.org/10.1016/j.eja.2011.11.004>.
- Tonitto, C., David, M.B., Drinkwater, L.E., 2006. Replacing bare fallows with cover crops in fertilizer-intensive cropping systems: a meta-analysis of crop yield and N dynamics. *Agric. Ecosyst. Environ.* 112, 58–72. <https://doi.org/10.1016/j.agee.2005.07.003>.
- Tosti, G., Benincasa, P., Farneselli, M., Tei, F., Guiducci, M., 2014. Barley-hairy vetch mixture as cover crop for green manuring and the mitigation of N leaching risk. *Eur. J. Agron.* 54, 34–39. <https://doi.org/10.1016/j.eja.2013.11.012>.
- Waggoner, M.G., 1989. Time of desiccation effects on plant composition and subsequent nitrogen release from several winter annual cover crops. *Agron. J.* 81, 236–241. <https://doi.org/10.2134/agronj1989.00021962008100020020x>.