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Intercropping and fertilization strategies to progress sustainability of organic cabbage and beetroot production

Sindhuja Shanmugam [a], Margita Hefner [a,b], Rodrigo Labouriau [c], Alessandra Trinchera [d], Koen Willekens [e], Hanne Lakkenborg Kristensen [a,*,1]

[a] Department of Food Science, Aarhus University, 8200 Aarhus N, Denmark
[b] Leibniz Institute of Vegetable and Ornamental Crops (IGZ), Großbeeren, Germany
[c] Department of Mathematics, Aarhus University, 8000 Aarhus C, Denmark
[d] Research Centre for Agriculture and Environment, Council for Agricultural Research and Economics (CREA), Rome, Italy
[e] Department of Crop Husbandry and Environment, Research Institute for Agriculture, Fisheries and Food, Merelbeke, Belgium

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ABSTRACT

Sustainably increasing organic vegetable crop productivity is needed to meet growing demands, considering replacement of conventional animal manures with alternative fertilizers. We investigated the effects of intercropping (IC) and different organic fertilization strategies, and their interactions, on the plant-soil system. A 2-year IC field experiment with white cabbage and beetroot was conducted with two compost-supplemented fertilization strategies (animal-based AF+C; plant-based PF+C) and one control with pig slurry (CONT). Root growth was measured with the minirhizotron method. Overall productivity of intercropping (IC) was lower or similar to that of monocropping (MC) systems with a land equivalent ratio of 0.8 in 2018 and 1.0 in 2019. IC affected rooting intensity in only few soil layers: at harvest (2018), beetroot IC had higher rooting intensity compared to beetroot MC in 0.25–0.75 m soil layer. Mycorrhizal colonization of beetroot roots was increased by 37 % under IC. CONT crops had the highest yield and nitrogen (N) accumulation in 2018. In 2019, yield, N and phosphorous (P) accumulation and soil enzyme activity were higher in the PF+C and CONT conditions than with AF+C. Potential N mineralization was 24–37 % higher under PF+C compared to CONT and AF+C, whereas hot water extractable P was highest under animal-based fertilization strategies (CONT: 8.66 mg kg^{-1}, AF+C: 8.56 mg kg^{-1}) compared to PF+C: 7.99 mg kg^{-1}. Benefits of productivity and N-use-efficiency from complementary root growth and resource use were not found in cabbage-beetroot IC. Instead, displacement of sowing/planting dates in the second year decided the dominating species, supported by mycorrhiza in beetroot. This management practice reduced the level of competition and increased the overall productivity of the IC system compared to 2018. The plant-based fertilization strategy had higher soil fertility as indicated by potential N mineralization and similar P use efficiency and can replace pig slurry. The methods of IC and fertilization strategy interacted only on potential N mineralization. Long-term improvements are expected with compost-supplemented fertilization strategies owing to their high organic carbon and N inputs.

1. Introduction

Nitrogen (N) is an important nutrient for sustaining productivity of vegetables, which often have a high N demand. In organic farming, this demand can be met by application of green manures, animal manures, compost, and other organic fertilizers. However, it is particularly difficult to predict how much and when N will be released from organic fertilizers resulting in a lower nitrogen use efficiency (NUE) and N losses to the environment through nitrate leaching and ammonia volatilization (Lim et al., 2018). Nitrogen availability is a major yield limiting factor in

Abbreviations: AF+C, Animal-based fertilization with compost; CONT, Animal-based fertilization without compost; IC, Intercropping; LER, Land equivalent ratio; MC, Monocropping; N, Nitrogen; NUE, Nitrogen use efficiency; NUpE, Nitrogen uptake efficiency; P, Phosphorus; PF+C, Plant-based fertilization with compost; PMN, Potential N mineralization; Root $intensity_{mod}$, Modified root intensity; HWC, Hot water extractable carbon; HWP, Hot water extractable phosphorus.

* Correspondence to: Department of Food Science, Aarhus University, Agro Food Park 48, 8200, Aarhus N, Denmark.
E-mail address: hanne.kristensen@food.au.dk (H.L. Kristensen).
[1] orcid.org/0000-0002-9200-1849

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organic cropping systems and the yield gap between organic and conventional agriculture can be as high as 33 % for some vegetable crops (Seufert et al., 2012). These reduced yields translate into greater N loss per unit product for organic agriculture compared to conventional agriculture (Reganold and Wachter, 2016). Given the increasing demand for organic vegetables, there is a pressing need for intensification of organic production systems which is sustainable and practiced in a way that maintains and improves long-term soil fertility.

Soil fertility and N use can be optimized by ensuring sufficient and preventing excessive N availability during the cropping season with the implementation of fertilization strategies that combine organic sources with complementary qualities. For example, organic fertilizers with a high carbon input, soil improving qualities and slow release of N (e.g., compost, farm yard manure-straw mixtures) may be combined with fertilizers with fast release of N (e.g., clover, pig slurry) to augment nutrient availability (Canali et al., 2012). Organic sources with a low biodegradability, such as compost, serve as a soil improver besides as a nutrient source with benefits such as increasing water holding capacity, nutrient availability, and microbial activity in soils (Lim et al., 2018; Luo et al., 2018). Composts made from plant residues have been shown to improve microbial activity, soil quality and a steady nutrient release (Tejada et al., 2009). Soil enzyme activities such as β-glucosidase and dehydrogenase can be an indicator of soil fertility through improved nutrient cycling and organic matter decomposition and are responsive to agricultural management practices such as organic amendment addition and tillage practices (Adetunji et al., 2017). Organic amendments with high P content can increase the soil phosphorus (P) availability and a risk for P leaching might be indicated by hot water extractable phosphorus (HWP) (Nest et al., 2015). Besides animal-based organic amendments, plant-based soil improvers and fertilizers can be produced on-farm in accordance with organic farming principles, making the production independent from conventional livestock sources and thus increasing the credibility of organic certified production (Oelofse et al., 2013).

Although there has been substantial research focused on organic amendments such as composts, green manures, and livestock manures (Baldi and Toselli, 2013; Canali et al., 2012; Ros et al., 2007; Tejada et al., 2009), little is known about the effects of plant-based soil improvers and fast N releasing fertilizers, particularly in combination, on crop growth and soil fertility. When applied together, two or more organic amendments with diverse chemical properties may complement each other's effects on crop yield, NUE and soil fertility through increased nutrient availability and C input (Tully and McAskill, 2020).

Complementary resource use and productivity can be increased in intercropping (IC), which is the practice of growing two or more crops together in the same field for all or part of their growing seasons (Shanmugam et al., 2022). Benefits under IC can be attained through niche separation, facilitative belowground and aboveground interactions, increased soil microbial population and biodiversity, improved soil fertility, disease, pest and weed suppression, relative to monocropping (MC) (Mariela et al., 2016; Schröder and Köpke, 2012; Xie and Kristensen, 2017). However, IC has also been reported to lead to unbalanced growth of crops, e.g. that only one high-quality product is ultimately harvested (Stavridou et al., 2012). Belowground interactions in IC systems may be decisive of complementary resource use and productivity due to inter-species competition or facilitation. Plants' spatial root distribution is modulated by root interactions and growth patterns of the neighboring plant (Schröder and Köpke, 2012; Shanmugam et al., 2022).

To the best of our knowledge, no studies have investigated interactions and additive effects from IC and fertilization strategies with the aim to identify ways to increase system productivity and sustainability. Thus, we carried out a 2-year field experiment (2018–2019) to investigate the effects of IC of white cabbage (*Brassica oleracea* L. var. *capitata* f. *alba*) and beetroot (*Beta vulgaris* L.) grown under different organic fertilization strategies on N dynamics, soil fertility, root growth, and yield parameters. With this experiment, we tested the following five hypotheses. (1) IC increases system productivity, relative to MC, through improved nutrient use efficiency. (2) IC influences on root growth and nutrient uptake leading to complementary resource use. (3) Organic fertilization is improved by combining a fast N releasing fertilizer with a slow N releasing compost, thereby improving crop yield, root growth, nutrient availability and C input to the system, compared to the use of only a fast N releasing fertilizer. (4) The combined plant-based fertilization strategy can maintain crop yield at levels comparable to those obtained with an animal-based fast releasing fertilizer. (5) The implementation of two management methods of IC and fertilization strategy interact by improving soil fertility and crop yield through increased soil microbial activity and mycorrhizal colonization.

2. Materials and methods

2.1. Field site

A 2-year field experiment was conducted at the Årslev research center in Denmark (10° 27′E, 55° 18′N) during the years of 2018 and 2019. The field has been managed organically since 2013 according to Danish regulations without the use of any synthetic pesticides or fertilizers. The field has a sandy loam soil type (Typic Agrudalf). The top soil layer (0–0.25 m) was found to have the following characteristics: 1.3 g kg^{-1} of total N, 30 mg kg^{-1} P_2O_5 (P extracted with 0.5 M $NaHCO_3$, Olsen P method), 155 mg K_2O (K extracted with 0.5 M CH_3COONH_4 and 3 mM LiCl, flame photometry [768 nm]) and 52 mg Mg (Mg extracted with 0.5 M CH_3COONH_4 and 3 mM LiCl, atomic absorption spectroscopy [285 nm]), and a soil pH_{CaCl2} of 6.3. The soil texture was as follows, by layer: at a depth of 0–0.25 m, 13 % clay, 15 % silt, 70 % sand, and 1.9 % organic matter; at a depth of 0.25–0.5 m, 15 % clay, 15 % silt, 69 % sand, and 1 % organic matter; at a depth of 0.5–1.0 m, 19 % clay, 13 % silt, 68 % sand, and 0.4 % organic matter; and at a depth of 1.0–2.5 m, 18 % clay, 15 % silt, 67 % sand, and 0.2 % organic matter. The average air temperature and cumulative precipitation were 12.8 °C and 377 mm during 2018 and 10.6 °C and 647 mm during 2019. The weather conditions at the experimental site are summarized in Fig. 1.

2.2. Experimental design

Cabbage (cultivar Storage no. 4) and beetroot (cultivar Forono) were grown separately or in the same plot, resulting in three cropping systems, namely cabbage MC, beetroot MC, and intercropping (IC). In the IC condition, cabbage and beetroot were grown in alternating rows, following a replacement design. The experiment followed a completely randomized split-plot design with four replicates of each fertilizer treatment and cropping system, with the former allocated to main-plots and the latter to sub-plots. The main-plot size was 14.4 × 10 m and the

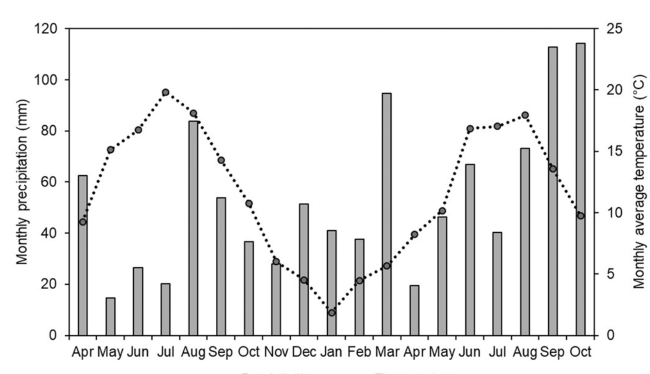

Fig. 1. Monthly average temperature and cumulative precipitation during the field experiment (April 2018–October 2019).

sub-plot size was 4.5 × 10 m which included nine crop rows. Row spacing was 0.5 m and plant spacing was 0.35 m and 0.04 m for cabbage and beetroot, respectively. In both years, the soil was worked with a cultivator and bed former to a depth of 0.2 m before sowing/planting. The crop grown in 2017, prior to this experiment, was spring barley (*Hordeum vulgare* L.) with an undersown grass-clover mixture, which was incorporated in November 2017. In October 2018, after the cabbage and beetroot harvest, hairy vetch (*Vicia villosa* L.) and corn cockle (*Agrostemma githago* L.) were sown in a mixture, grown as a catch crop, and incorporated on May 14th, 2019. The timing of management operations is outlined in Table 1. In 2019, crop plots were interchanged so that cabbage MC plots became beetroot MC plots and vice versa. In IC plots, crop rows were interchanged.

Each cropping system was paired with three fertilization strategies: animal-based fertilization (slurry and manure) without compost served as the control strategy (CONT); animal-based fertilization with compost (AF+C); and plant-based fertilization with compost (PF+C). Each fertilization strategy involved several fertilizer sources. These fertilizer sources and their time of application are described in Table 2. In 2018, chicken manure, lupine seeds, and commercial food residue fertilizer were applied as a split dose one month after the first fertilizer application to meet crop requirements. Because there was sufficient N availability from spring soil mineral N, catch crop, and the initial fertilizer application, the split dose was not necessary in 2019. The nutrient content of each fertilizer is shown in Table 3.

Fertilization was planned such that it would meet the recommended quantities of fertilizer N according to Danish fertilizer recommendations (180 kg N ha^{-1} for cabbage; 160 kg N ha^{-1} for beetroot; and an average of 170 kg N ha^{-1} for the IC system). Potential N mineralization (PMN) from the soil (measured by aerobic incubation, described in Section 2.3) and accumulated N in catch crops at the time of incorporation (mid-May 2019, Section 2.4) were taken into account and rest of the N requirement was given through fertilizers. The N and C constituents in each fertilizer treatment are shown in Table 3. Due to a difference between expected and analyzed values, fertilizer N application was higher in 2019 than in 2018, especially for PF+C (Table 4). The different fertilization strategies were each applied on the same plots in the two consecutive experimental years. All plots were sprinkle-irrigated with cumulative 275 mm (11 irrigations) in 2018 and 125 mm (6 irrigations) in 2019. Cabbage plots were sprayed with 1 L ha^{-1} of *Bacillus thuringiensis* subsp. *kurstaki* (Dipel DF) and *Bacillus thuringiensis* var. azawi (Turex) once in 2018 and twice in 2019, for pest control against diamondback moth and large white butterfly larvae.

Table 1
Dates of sampling and management operations in the field experiment.

	2018	2019
Soil sampling (0–2.5 m), in spring	Apr 20	Apr 11
Cabbage sowing in greenhouse	Apr 19	May 22
Beetroot sowing in field	May 16	June 06
Cabbage transplanting to field	May 24	June 25
Root filming in minirhizotrons	Jul 3, Aug 1, Sep 21	Jul 26, Aug 24, Oct 22
Soil sampling for PMN* (0–0.25 m)	Jul 18	Jun 17, Aug 09, Oct 29
Soil sampling, mid-season		Aug 09
Mid-season plant sampling	Jul 17	Aug 06
Mycorrhizae soil sampling (0.1–0.35 m)	–	Aug 29
Cabbage harvest	Sep 14	Oct 10
Beetroot harvest	Sep 26	Oct 14
Soil sampling (0–2.5 m), harvest	Sep 20	Oct 24
Weeding	Jun 7, Jun 21	Jun 27, Jul 4, Aug 1
Pest control	Jun 15, Jul 2, Jul 26	Jul 5, Jul 15, Aug 23

*PMN, potential N mineralization

Table 2
Sources of fertilizer used in the three fertilization strategies and the time of application.

Fertilizers	Time of application	
	2018	2019
CONT		
Pig slurry	Apr 19	May 16
Chicken manure	Jun 14	NA
AF+C		
Garden waste compost[a]	Apr 23, Oct 3	–
Deep litter (cattle)	May 14	May 27
Biofiber (solid biofiber fraction from anaerobic digestion)	May 14	May 27
Monterra 13 (commercial food residue fertilizer)	Jun 14	NA
PF+C		
Garden waste compost[a]	Apr 23, Oct 3	–
Clover silage (2018)/fresh clover(2019)	Apr 23	May 29
Crushed lupine seeds	Jun 14	NA

[a] Produced by aerobic pile composting (Klintholm I/S), NA=not applied, –=applied in October 2018. CONT, pig slurry; AF+C, animal-based fertilizers and compost; and PF+C, plant–based fertilizers and compost.

2.3. Soil sampling, potential N mineralization, and enzyme activity

Soil samples were taken at depths of 0–0.25 m, 0.25–0.5 m, 0.5–1.0 m, 1–1.5 m and 1.5–2.5 m at the start and end of the growing season with a machine-driven soil piston auger that had a 14-mm inner diameter. Mid-season soil samples were taken from the top soil layer (0–0.25 m) with a hand-driven soil auger that had a 15-mm inner diameter. Twelve subsamples within each plot were taken to obtain one soil sample per depth interval, which was mixed well and frozen at − 18 °C until the analyses were conducted. In preparation for analysis, each frozen soil sample was thawed and a 100-g aliquot (fresh weight) was extracted with 1 M KCl for 1 h (1 soil: 2 solution). The extractant was centrifuged and the supernatant solution was analyzed for NH_4^+ and NO_3^- by standard colorimetric methods in an AutoAnalyzer 3 (Bran + Luebbe, Germany).

For determination of PMN, top-soil-layer (0–0.25 m) samples were taken once in 2018 and three times in 2019 (Table 1) with the hand-driven auger as for mid-season mineral N samples. Field-moist soil was sieved using a 5-mm sieve and incubated at 25 °C for 4 wks in 500-ml containers covered with polyethylene. Moisture content was kept constant by adding water to compensate for weight loss. PMN was calculated by subtracting the initial mineral N content at the start of the incubation from that at the end of the incubation (Hefner et al., 2019).

In 2019, the activities of the soil enzymes β-glucosidase and dehydrogenase were analyzed as described by Moeskops et al. (2010); these enzyme activities were interpreted as indicators of soil microbial activity before (May 16, 2019) and after (June 17, 2019) fertilization. For β-glucosidase activity analyses, 1 ml 25 mM p-nitrophenyl-β-D-glucoside solution (PNG) and 4 ml of modified universal buffer were added to 1 g soil. The analysis was performed with two replicates and one control without PNG solution. The samples were incubated at 37 °C for 1 h, after which PNG solution was added to the control samples. After incubation, 1 ml 0.5 M $CaCl_2$ and 4 ml Tris-buffer (pH 12) were added to each sample. The extractant was then filtered immediately through a grade-5 Whatman filter. The optical density (OD) of released p-nitrophenol in the extract was measured by a spectrophotometer (Shimadzu, UV-1700) at 400 nm. β-glucosidase activity was calculated as the difference between values obtained for PNG-incubated samples and the control.

For dehydrogenase activity analysis, 2 ml of 3% triphenyltetrazolium chloride solution (TTC) and 2 ml of Tris-buffer pH 7.6 were added to 5 g samples of soil; control samples consisted of 5 g soil combined with 4 ml of only Tris-buffer. After a 24-h incubation at 37 °C, 20 ml of methanol was added to each sample, and the samples were shaken at 125 rpm for 2 h. Subsequently, each sample was filtered

Table 3
Nutrient content in fertilizers and compost on a dry-weight basis.

Fertilizers and compost	Dry matter (g kg^{-1})	N (g kg^{-1})	Organic C (g kg^{-1})	C:N ratio	P (g kg^{-1})	K (g kg^{-1})	S (g kg^{-1})
2018							
Pig slurry	26	117	383	3	25	60	10
Chicken manure	900	43	379	9	8	27	5
Garden waste compost	576	8	107	14	–	–	–
Deep litter manure	255	25	491	20	7	34	4
Biofiber	327	28	376	13	37	12	7
Food residue fertilizer (Monterra 13)	924	122	452	4	3	13	15
Clover silage	277	30	412	14	4	27	2
Lupine seeds	873	43	383	9	4	9	1
2019							
Pig slurry	10	217	305	1	15	133	8
Garden waste compost	740	6	83	13	0.2	4	–
Deep litter manure	215	37	474	13	6	34	5
Biofiber	286	30	390	13	40	14	7
Fresh clover	161	37	430	12	3	25	2

Table 4
Quantity of N and C input by fertilization strategy based on fertilizer source analyses.

Fertilizer	N input (kg ha^{-1})			C input (Mg ha^{-1})		
	Cabbage MC	Beetroot MC	IC	Cabbage MC	Beetroot MC	IC
2018						
CONT						
Pig slurry	61	61	61	0.21	0.21	0.21
Chicken manure	32	22	12	0.29	0.20	0.11
Total	*93*	*82*	*72*	*0.50*	*0.4*	*0.31*
AF+C						
Garden waste compost	12	12	12	0.16	0.16	0.16
Deep litter manure	32	18	25	0.65	0.38	0.51
Biofiber	21	21	21	0.28	0.28	0.28
Food residue fertilizer (Monterra 13)	20	13	17	0.07	0.05	0.06
Total	*84*	*64*	*74*	*1.16*	*0.86*	*1.01*
PF+C						
Garden waste compost	12	12	12	0.16	0.16	0.16
Clover silage	61	44	52	0.85	0.61	0.73
Lupine seeds	11	8	10	0.10	0.07	0.09
Total	*83*	*63*	*73*	*1.10*	*0.84*	*0.97*
2019						
CONT						
Pig slurry, total	*97*	*97*	*97*	*0.14*	*0.14*	*0.14*
AF+C						
Compost	47	47	47	0.61	0.61	0.61
Deep litter manure	75	68	73	0.97	0.87	0.94
Biofiber	17	17	17	0.22	0.22	0.22
Total	*139*	*132*	*137*	*1.80*	*1.71*	*1.77*
PF+C						
Compost	47	47	47	0.62	0.62	0.62
Fresh clover	141	154	145	1.63	1.79	1.68
Total	*187*	*201*	*192*	*2.24*	*2.40*	*2.30*

CONT, pig slurry; AF+C, animal-based fertilizers and compost; and PF+C, plant-based fertilizers and compost.

through grade-5 Whatman filters and each extract was diluted with methanol to a final volume of 50 ml. The rate of TTC reduction to triphenyltetrazolium formazan was estimated by spectrophotometer measurement of OD at 485 nm. Dehydrogenase activity was determined as the difference between samples with versus without added TTC.

2.4. Plant sampling and analysis

Fresh cabbage heads, beetroot (taproot) and plant residues of both crops were hand-harvested from two 3-m long rows, rinsed to remove soil, and weighed to obtain yield (fresh weight) and total aboveground biomass (dry weight). The marketable yield was assessed based on product size (cabbage head > 500 g or beetroot > 60 g) and pest incidence. Yield was calculated as kg per meter row in order to directly compare the productivity between the monocropping and intercropping systems, irrespective of the plant population per hectare. The N contents of plant parts (cabbage heads, stem and leaf residues, beetroots and leaf residues) were assessed by the VDLUFA method (VDLUFA, 1991). Briefly, plant parts were chopped, mixed well, oven-dried at 80 °C for 20 h, and burned at 900 °C. N content was determined by a Truspec® CN analyser (LECO, St. Joseph, MI).

2.5. Root growth

Root growth was determined (CONT and PF+C) by the minirhizotron method, where transparent 3-m-long plastic tubes were inserted in the field at a 30° angle from vertical to a depth of 2.4 m (Kristensen and Thorup-Kristensen, 2007). After sowing/planting, two tubes were inserted within each plot (one tube per row) and in three blocks. In IC plots, one tube was inserted in one row of each crop. Two counting grids (40 × 40 mm) were drawn on the upper side of the tube. The tubes were inserted in the field after transplanting/sowing of the crops and filmed three times during the crop growth period (Table 1) with a mini video camera (resolution, 800 × 600 pixels). Root intersections were counted and analyzed in these films as described by Hefner et al. (2019). Modified root intensity (root intensity$_{mod}$) was registered as the number of roots crossing grid lines. Root frequency was registered as the presence or absence of any root in each grid. Root intensity$_{mod}$ was summed for each 0–0.25 m soil layer and root frequency was expressed as percentage of grids occupied by roots out of the total number of grids in the layer. The deepest root registered in the grids of each tube was used to calculate the average root depth of the crops. All roots registered in the tube in the beetroot row were counted as beetroot roots and all roots registered in the tube in the cabbage row were counted as cabbage roots. Under IC, beetroot and cabbage roots were attempted distinguished visually, using the pink colored beetroot roots and white colored cabbage roots. However, the distinction was not completely clear as young beetroot roots were found to be white in several occasions.

2.6. Hot water extractable carbon (HWC) and HWP

A top soil layer (0–0.25 m) sample from each plot at the time of harvest was oven-dried at 70 °C and passed through a 2-mm sieve. The soil that did not pass was crushed until all soil had passed through the sieve. HWC and HWP were extracted following a modified method of Sparling et al. (1998). Briefly, a 5-g air-dried sample was extracted with 25 ml of demineralized water for 16 h in a hot water bath (70 °C). Each soil suspension was centrifuged and its C and P levels were determined by a Vista-Pro® CCD simultaneous IVP-OES machine (Varian).

2.7. Root mycorrhizal colonization

At harvest in 2019, root samples were collected with an auger from the 0.1–0.35-m soil layer of each plot ca. 10 cm from plant axes both in the row and inter-row. After shaking off of residual soil, root pieces were rinsed with tap water on a 0.5-mm sieve and then stained for 5 min with methyl blue (0.05 % w/v) dissolved in lacto-glycerol solution (1:1:1 lactic acid, glycerol, and water). Stain solution was removed by placing roots in distilled water for 3 min. Ten thin (<0.5 mm diameter) 1-cm-long root segments were cut 5–15 mm from the root tip and fixed on a slide with Canada balm. IC root slides were made separately for each crop. The root segments, in which mycorrhizal fungi vesicles and hyphae were stained blue, were observed under a light microscope (E100, Nikon) at 40× magnification. The magnified intersections method was used in determining the percentage of each segment that is occupied by arbuscular mycorrhizal fungal structures (McGonigle et al., 1990). Mycorrhizal colonization of each root fragment was scored on a 0–5 scale according to Trinchera et al. (2019) and as follows; 0 = no AMF structures within the root segment; 1 = structures occupy < 1 % of the root segment; 2 = structures occupy < 10 % of the root segment; 3 = structures occupy < 50 % of the root segment; 4 = structures occupy more than 50 % of the root segment; 5 = structures occupy more than 90 % of the root segment.

2.8. Calculations and statistical analysis

N accumulation in aboveground biomass and in beetroot was calculated based on plant dry weight and N content. N balance was calculated as N input [fertilizer N (including compost) + soil mineral N at the start (0–0.25 m) + N coming from catch crop biomass] – N output (N accumulation in aboveground biomass + soil mineral N at harvest) (Kyllingsbæk and Hansen, 2007). N uptake efficiency (NUpE) was calculated as the ratio between accumulated N in the aboveground biomass and total available N (soil mineral N at harvest + N accumulation in aboveground biomass)(Xu et al., 2012).

Land equivalent ratios (LERs) were calculated according to Mead and Willey (1980) using the formula as follows:

$$LER = \frac{\text{cabbage yield (IC)}}{\text{cabbage yield (MC)}} + \frac{\text{beetroot yield (IC)}}{\text{beetroot yield (MC)}} \quad (1)$$

Total yield (fresh weight) was used for LER calculations. A LER > 1 (or < 1) indicated that the IC system needed less (or more) area of land than the MC systems to yield the same. Partial LER's of cabbage and beetroot were indicated by the first and second part of the Eq. 1, respectively. LERs for aboveground N accumulation (LER-N) were calculated as described by Willey (1979), wherein yield is replaced by plant N accumulation in Eq. 1.

Root growth was analyzed as described by Hefner et al. (2019). Separate analyses were performed for each year and depth zone. Root frequency was analyzed with a generalized linear mixed model (GLMM) defined with a binomial distribution, the logistic link function, fixed effects for cropping system, fertilization treatment and their interaction, and two independent Gaussian random components representing the blocks and the tubes.

Root intensity$_{mod}$ was analyzed with a GLMM obtained with the Poisson distribution, the logarithmic link function, fixed effects for cropping system, fertilization treatment and their interaction, and an offset containing the logarithm of the number of observations in each combination of cropping system, fertilization strategy and block. The model contained also two independent Gaussian random components representing the blocks and the tubes. Estimates of the fixed effects of these GLMMs are the mean number of roots crossing reference lines in each observational window, a value that is proportional to the length of visible roots in each observational window (Pelck and Labouriau, 2020). The modeled estimates of the root intensity$_{mod}$ represent the mean number of times the observed roots crossed the reference lines of an observational window. As explained in Hefner et al. (2019), (technical appendix) and Pelck and Labouriau (2020), when using stochastic geometric arguments it can be shown that the average number of crosses in the present analysis is proportional to the length of the visible roots.

Statistical analyses of all other variables were analyzed using a GLMM defined with the Gamma distribution, identity link function, fixed effects given by a combination of cropping system and fertilization strategy, and a Gaussian random component representing the block. Likelihood ratio tests were used for determining the presence of interaction, additivity, or significance of fixed effects. Post hoc analyses were performed using the R-package postHoc (Labouriau, 2020) with correction of *P*-values for multiple testing using the method of control of the false discovery rate (FDR), (Benjamini and Yekutieli, 2001). The data were analyzed in R software (version 3.6.3, R Core Team, 2020).

3. Results

3.1. Crop yield and competition

Calculated as kg per meter row, cabbage total and marketable yield were higher under IC than under MC in 2018, but lower under IC in 2019 (Table 5). Conversely, beetroot total and marketable yields (kg m^{-1} row) were lower under IC than under MC in 2018 but higher under IC in 2019 (Table 5). In the IC system, partial LER (per crop) averaged across fertilization strategy was lower for beetroot (0.20) than cabbage (0.63) in 2018 but was lower for cabbage (0.37) than beetroot (0.59) in 2019. The overall LER for IC systems was 0.83 in 2018 and 0.96 in 2019.

Concerning fertilization strategies, cabbage yield was higher under CONT than under AF+C and PF+C in 2018, whereas the yield was similar for all fertilization strategies in 2019 (Table 5). Beetroot total yield was highest under CONT in 2018, while it was higher under CONT and PF+C than AF+C in 2019. The lowest marketable yield of beetroot was registered under PF+C in 2018 and AF+C in 2019 (Table 5).

3.2. Biomass and nutrient accumulation

In 2018, the biomass (dry weight) achieved was greatest for beetroot MC, followed by cabbage MC and IC in 2018. In 2019, similar biomasses were produced for all cropping systems (Table 6). Cabbage MC had higher N accumulation in the total aboveground biomass than beetroot MC and IC in 2018. However, in 2019, beetroot MC and IC had higher N accumulation than cabbage MC (Table 6). On average, across fertilization strategies, the total biomass LER-N was 0.86 in 2018 and 1.00 in 2019 (results not shown). In 2018, beetroot N concentration was reduced under IC (9.02 g kg^{-1}) compared to beetroot MC (10.04 g kg^{-1}) ($P = 0.046$). In 2019, cabbage head N concentration was reduced under IC (18.85 g kg^{-1}) compared to cabbage MC (20.85 g kg^{-1})(results not shown; $P = 0.007$). Both cabbage and beetroot MC systems had higher P accumulation levels than the IC system in 2018; there was no difference among cropping systems in 2019 (Table 6). With the exception of beetroot P concentration, which was higher under IC (2.59 g kg^{-1}; $P < 0.001$ vs. MC) in 2018, cropping system did not affect cabbage-head or beetroot P concentrations in either year (results not shown).

Among fertilization strategies, the highest N accumulation was found under CONT in 2018. In 2019, N accumulation was higher under CONT and PF+C than AF+C (Table 6). In 2018, N concentration was similar across fertilization strategies in cabbage heads and beetroots, but the cabbage residue N concentration was highest under the CONT (results not shown; $P = 0.002$). In 2019, N concentrations of cabbage heads and beetroots were higher under CONT and PF+C than AF+C. P

Table 5
Total and marketable yields (fresh weight) by cropping system and fertilization strategy, calculated per meter crop row.

	Cabbage				Beetroot			
	Total yield (kg m^{-1} row)		Marketable yield (kg m^{-1} row)		Total yield (kg m^{-1} row)		Marketable yield (kg m^{-1} row)	
	2018	2019	2018	2019	2018	2019	2018	2019
Cropping system								
MC	4.6^{a}	2.7^{b}	4.1^{a}	2.5^{b}	4.5^{b}	3.8^{a}	4.2^{b}	3.7^{a}
	(4.3–4.9)	(2.2–3.3)	(3.8–4.6)	(1.9–3.2)	(3.7–5.2)	(3.4–4.2)	(3.3–5.0)	(3.3–4.1)
IC	5.9^{b}	2.0^{a}	5.5^{b}	1.6^{a}	2.2^{a}	4.2^{b}	1.7^{a}	4.1^{b}
	(5.6–6.2)	(1.5–2.4)	(5.0–6.0)	(1.1–2.1)	(1.6–2.7)	(3.8–4.6)	(1.1–2.3)	(3.7–4.5)
Fertilization strategy								
CONT	4.6^{b}	2.7^{a}	4.2^{b}	2.5^{a}	4.5^{a}	3.8^{b}	4.2^{b}	3.7^{b}
	(4.3–4.9)	(2.2–3.3)	(3.8–4.6)	(1.9–3.2)	(3.7–5.2)	(3.4–4.2)	(3.3–5.1)	(3.3–4.1)
AF+C	3.6^{a}	2.4^{a}	3.4^{a}	2.3^{a}	3.7^{a}	2.7^{a}	3.6^{ab}	2.6^{a}
	(3.4–3.9)	(1.9–2.9)	(3.0–3.7)	(1.7–2.9)	(3.1–4.4)	(2.4–3.0)	(2.8–4.4)	(2.3–2.8)
PF+C	3.9^{a}	2.9^{a}	3.4^{a}	2.8^{a}	3.7^{a}	3.4^{b}	3.4^{a}	3.4^{b}
	(3.7–4.2)	(2.3–3.4)	(3.1–3.8)	(2.1–3.5)	(3.1–4.4)	(3.1–3.8)	(2.6–4.2)	(3.0–3.7)
P-value								
Cropping system	< 0.001	0.004	< 0.001	0.022	< 0.001	0.032	< 0.001	0.025
Fertilization strategy	< 0.001	n.s.	0.011	n.s.	n.s.	< 0.001	0.036	< 0.001
Cropping system × fertilization strategy interaction	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Estimates are given with 95 % confidence intervals in parenthesis (n = 4). Different lower-case letters indicate significant differences among treatments. Cropping systems: Cabbage MC, cabbage monocropping; Beetroot MC, beetroot monocropping; and IC, intercropping. Fertilization strategies: CONT, pig slurry; AF+C, animal-based fertilizers and compost; and PF+C, plant–based fertilizers and compost. N.s., not significant.

Table 6
Biomass (dry weight) and nutrient accumulation in total aboveground biomass by cropping system and fertilization strategy.

	Total biomass (Mg ha^{-1})		N accumulation (kg N ha^{-1})		P accumulation (kg P ha^{-1})	
	2018	2019	2018	2019	2018	2019
Cropping system						
Cabbage MC	13.9^{b}	10.8^{a}	218^{b}	225^{a}	37^{b}	31^{a}
	(12.8–15.0)	(9.8–11.8)	(202–234)	(196–255)	(34–41)	(28–34)
Beetroot MC	16.5^{c}	11.5^{a}	186^{a}	292^{c}	38^{b}	34^{a}
	(15.3–17.7)	(10.4–12.5)	(171–201)	(259–325)	(35–42)	(30–37)
IC	12.7^{a}	11.4^{a}	184^{a}	257^{b}	34^{a}	33^{a}
	(11.6–13.7)	(10.4–12.4)	(170–199)	(226–288)	(31–37)	(29–36)
Fertilization strategy						
CONT	16.5^{b}	11.5^{b}	186^{b}	292^{b}	38^{b}	34^{b}
	(15.3–17.7)	(10.4–12.5)	(171–201)	(259–325)	(35–42)	(30–37)
AF+C	14.4^{a}	9.4^{a}	143^{a}	211^{a}	34^{ab}	28^{a}
	(13.3–15.5)	(8.5–10.4)	(131–156)	(183–240)	(31–38)	(25–31)
PF+C	13.5^{a}	10.7^{ab}	134^{a}	252^{b}	31^{a}	31^{b}
	(12.3–14.6)	(9.7–11.7)	(121–147)	(222–283)	(28–34)	(27–34)
P-value						
Cropping system	< 0.001	n.s.	< 0.001	< 0.001	0.013	n.s.
Fertilization strategy	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Cropping system × fertilization strategy interaction	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Estimates are given with 95 % confidence intervals in parenthesis (n = 4). Different lower-case letters indicate significant differences among treatments. Cropping systems: Cabbage MC, cabbage monocropping; Beetroot MC, beetroot monocropping; and IC, intercropping. Fertilization strategies: CONT, pig slurry; AF+C, animal-based fertilizers and compost; and PF+C, plant–based fertilizers and compost. N.s., not significant.

accumulation in total biomass was lowest under PF+C in 2018, but lowest under AF+C in 2019 (Table 6). P concentrations of both cabbage and beetroot were similar for all fertilization strategies, with exception of beetroot residues in 2019, which had a higher P concentration under CONT than PF+C and AF+C (results not shown; $P < 0.001$).

3.3. Root growth

There were interactions between effects of cropping system and fertilization strategy on root intensity$_{mod}$ and root frequency. Within the interactions, we explain the effects of cropping system for each fertilizer treatment and the effects of fertilization strategy for each cropping system separately.

3.3.1. Effect of cropping systems

In 2018, cabbage MC had higher root intensity$_{mod}$ than beetroot MC in the 0–0.75-m, 0–1.5-m, and 0–1.25-m soil layers in July, in August, and in September, under fertilization strategies with (PF+C) and without compost inclusion (CONT); in August, this inter-crop difference was maintained down to 1.75-m depth under CONT (Fig. 2, Fig. 3). Greater root intensity$_{mod}$ was observed in July for cabbage IC than for cabbage MC within the 0.5–0.75-m soil layer under PF+C. However, beetroot IC had higher root intensity$_{mod}$ than beetroot MC in the 0.25–1.25-m and 0.25–0.75-m soil layers in August and September, respectively, under CONT (Fig. 2). Despite these differences in root intensity$_{mod}$, root frequency was similar across cropping systems, except in the 0–0.25-m soil layer at all observation dates and in the 0.75–1.25-m soil layer in August, where cabbage MC had the higher rooting frequencies than beetroot MC under CONT condition (results not shown).

During 2019, cabbage MC developed a greater root intensity$_{mod}$ than beetroot MC at a depth of 0–0.5-m in the months of August and October under CONT and in the 0.25–0.5-m soil layer in October under PF+C (Fig. 2, Fig. 3). Beetroot MC produced higher root intensity$_{mod}$ than cabbage MC in the 0.25–0.5-m and 1–1.25-m soil layers in July and

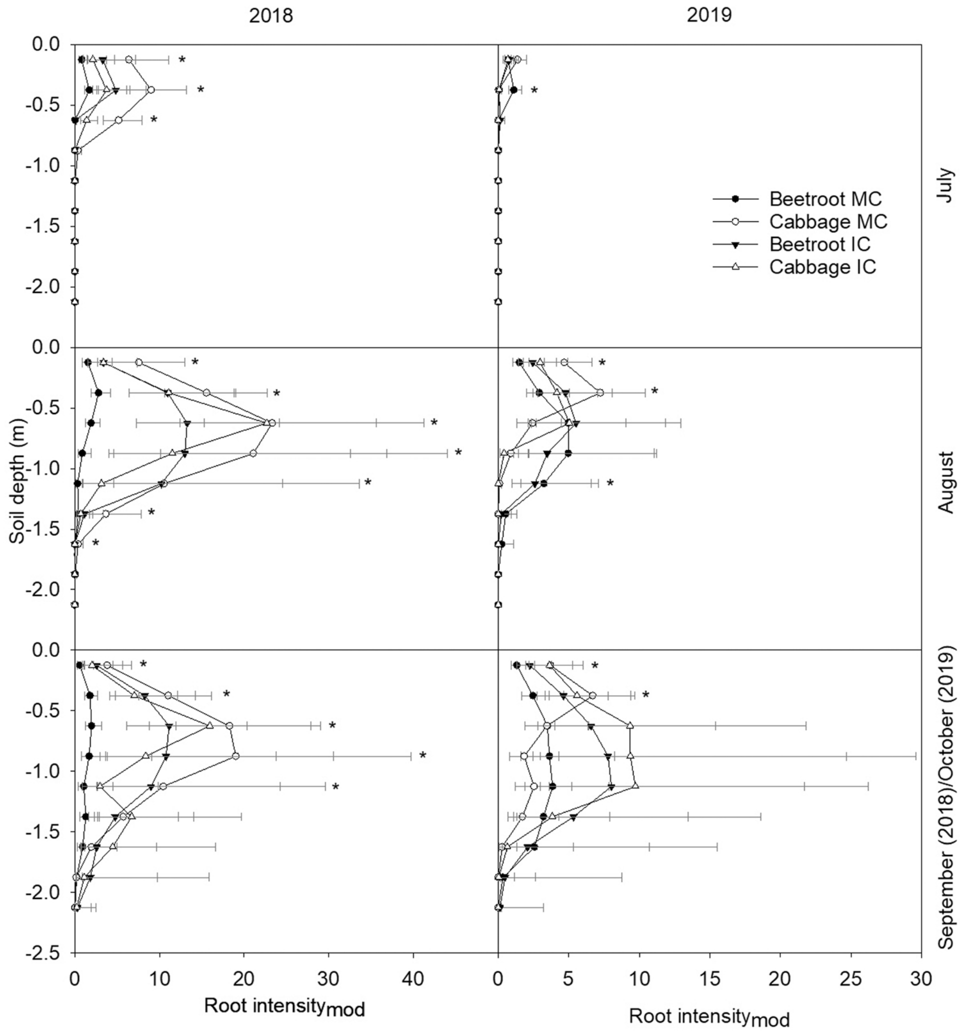

Fig. 2. Root $intensity_{mod}$ of cabbage and beetroot grown with pig slurry fertilization (CONT) in 2018 and 2019. MC, monocropping; IC, intercropping. Bars indicate 95% confidence intervals, n = 3; * $P < 0.05$ testing the significant difference between cropping systems in any given depth. Note the x-axis scale change between years.

August, respectively, under both fertilization strategies. Increased root $intensity_{mod}$ for IC plants, compared to respective MC systems, was not seen for either crop in any soil layer. Similar root frequencies were observed across all cropping systems under both fertilization strategies, with the exceptions of the 0–0.25-m soil layer in October (highest in cabbage MC) and the 0.75–1-m soil layer in August (highest in beetroot MC) under CONT (results not shown). Root depth did not differ significantly between cropping systems or between fertilization strategies, with the exception of cabbage roots reaching deeper depths in the CONT condition (1.9 m) than in the PF+C condition (1.7 m) in 2018. The average root depths of cabbage and beetroot were 2 m and 1.8 m in 2018 and 1.7 m and 2 m in 2019, respectively (results not shown).

3.3.2. Effect of fertilization strategies

Effects of fertilization strategies on root growth are described only for the time of harvest. Root $intensity_{mod}$ was higher under CONT than PF+C in the 0.75–2-m soil layer for beetroot IC in 2018 and for the 0.5–0.75-m soil layer for cabbage IC in 2019. In contrast, root $intensity_{mod}$ was higher under PF+C than in CONT in the 0.5–0.75-m soil layer for beetroot MC in 2018 and in those from the 0.75–2.0-m soil layer for cabbage MC in 2019 (results not shown).

3.4. N dynamics

Regarding cropping systems, at the time of harvest in both years i.e. September 2018 and October 2019, cabbage MC left lower residual soil mineral N (0.5–1.0 m soil depth in 2018 and 0–1.0 m soil depth in 2019) than beetroot MC. At the same time, residual soil mineral N in IC was similar to cabbage MC in 2018 and beetroot MC in 2019 (Fig. 4, left panel). However, in April 2019, cabbage MC (site of beetroot MC in 2018) had lower soil mineral N in the 0.5–1.5-m soil layer than beetroot MC (site of cabbage MC in 2018) and IC, whereas soil mineral N of IC was similar to that of beetroot MC in the 0.25–0.5- and 1.0–1.5-m soil layers. Similarly, during August 2019, beetroot MC (site of cabbage MC in 2018) had higher soil mineral N than cabbage MC and IC in the 0.5–1.5-m soil layer. No differences among cropping systems were found in the deepest soil layer of 1.5–2.5 m in September 2018, April 2019, or October 2019 (Fig. 4, left panel). Compared to the other cropping systems, a higher NUpE was found with cabbage MC in 2018 and with beetroot MC in 2019. In contrast, N balance was higher for beetroot MC in 2018 and for cabbage MC in 2019 (Table 7).

Regarding fertilization strategy, crops grown under CONT and PF+C left more soil mineral N in the 0–0.25-m layer in September 2018, in the 0.5–1.5-m layer in April 2019, in the 1.0–1.5-m layer in August 2019, and in the 0–0.25-m layer in October 2019 compared to crops grown under AF+C. Only in the 0.25–0.5-m soil layer in October 2019 was the

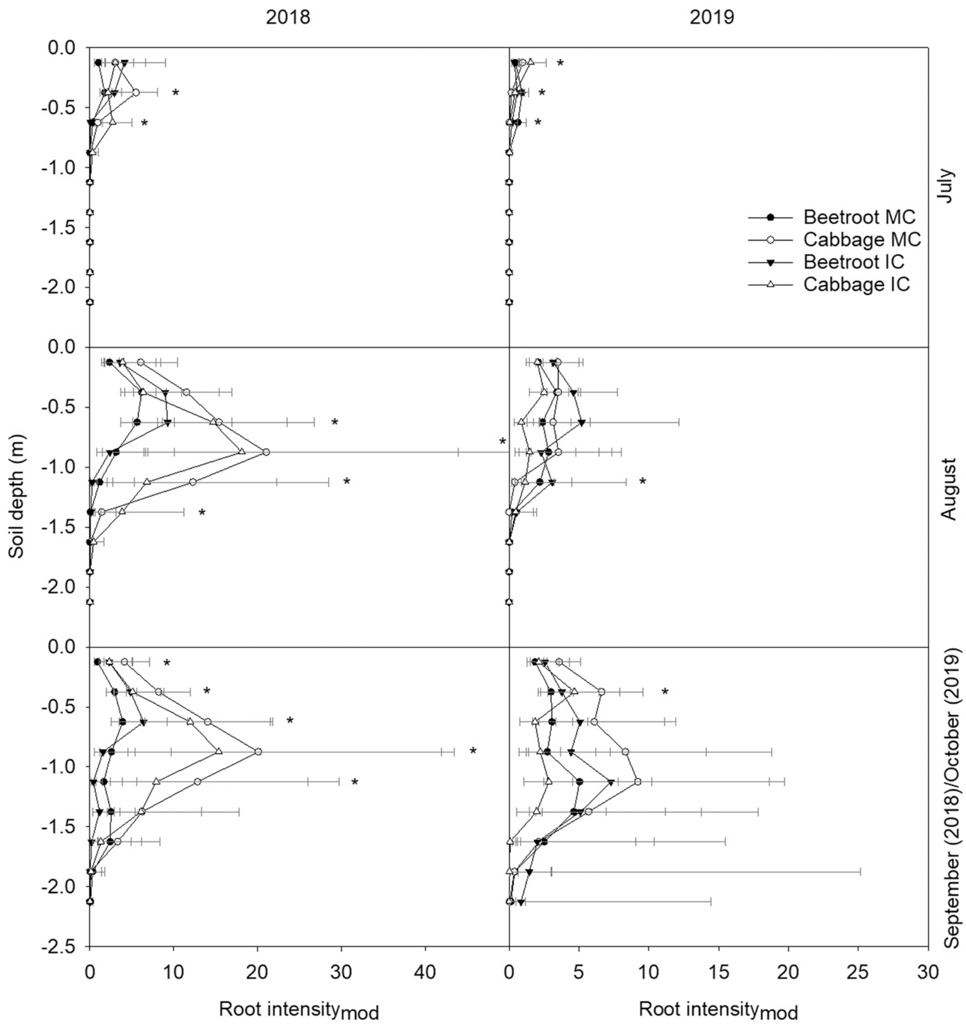

Fig. 3. Root $intensity_{mod}$ of cabbage and beetroot grown with a fertilization strategy of plant-based fertilizer and compost (PF+C) in 2018 and 2019. MC, mono cropping; IC, intercropping. Bars indicate 95% confidence intervals, n = 3. * $P < 0.05$ testing the significant difference between cropping systems in any given depth. Note the x-axis scale change between years.

soil mineral N content similar between the AF+C and CONT conditions, at which time soil mineral N content was lowest in the PF+C condition. At all other sampling times and sampling depths, no differences between fertilization strategies were found (Fig. 4, right panel). CONT had higher NUpE and lower N balance values than the other fertilization strategies in both years (Table 7).

3.5. Microbial activity, mycorrhizal colonization, HWC, and HWP

Among fertilization strategies, PMN was higher with PF+C fertilization than with CONT or AF+C fertilization in June, and a cropping system-fertilization strategy interaction was detected for August 2019 (Table 8). Mycorrhizal colonization intensity was not affected by fertilization strategy. However, beetroot IC (15.7 %) had higher mycorrhizal colonization intensity than beetroot MC (11.5 %) ($P < 0.001$). Even at the non-mycorrhizal plant of cabbage, a similar trend was observed, although mycorrhizal colonization intensity in IC systems did not exceed 7% (Fig. 5). Dehydrogenase activity was not affected by cropping system or fertilization strategy before or after fertilization (results not shown). However, β-glucosidase activity was higher in CONT and PF+C soil samples than in AF+C soil samples at both times (Fig. 6). These fertilization strategies also resulted in higher HWC values at the time of harvest in 2019 (Fig. 7). HWP was higher in MC system soil samples than in IC samples, and HWP was also higher in CONT and AF+C samples than in PF+C samples (Fig. 7).

4. Discussion

4.1. Effects of IC on productivity

The present study showed that IC affected cabbage and beetroot yields differently between the two study years. The total yields obtained for cabbage MC (51–83 Mg ha^{-1}) and beetroot MC (49–85 Mg ha^{-1}) were slightly higher than the yields reported in a prior Danish organic study (cabbage, 44–57 Mg ha^{-1}; and beetroot, 37–50 Mg ha^{-1})(Hefner et al., 2019). The overall higher yields and root growth that we observed in 2018, relative to 2019, could be explained by a warmer summer coupled with sufficient irrigation (Fig. 1). Based on yield per meter row values, cabbage was more competitive and productive under IC than MC in 2018, whereas beetroot yield was reduced by half in IC plots compared to beetroot MC plots (Table 5). Our observations of a low partial LER and a reduced N concentration in beetroot biomass under the IC system suggest that IC-associated competition may have reduced overall land use efficiency and productivity of crops that year. Sometimes, IC is associated with productivity compensation, such that one crop has higher yields while the other crop suffers a yield reduction due to belowground, aboveground, or total inter-species competition. For example, when broccoli was intercropped with cauliflower in a

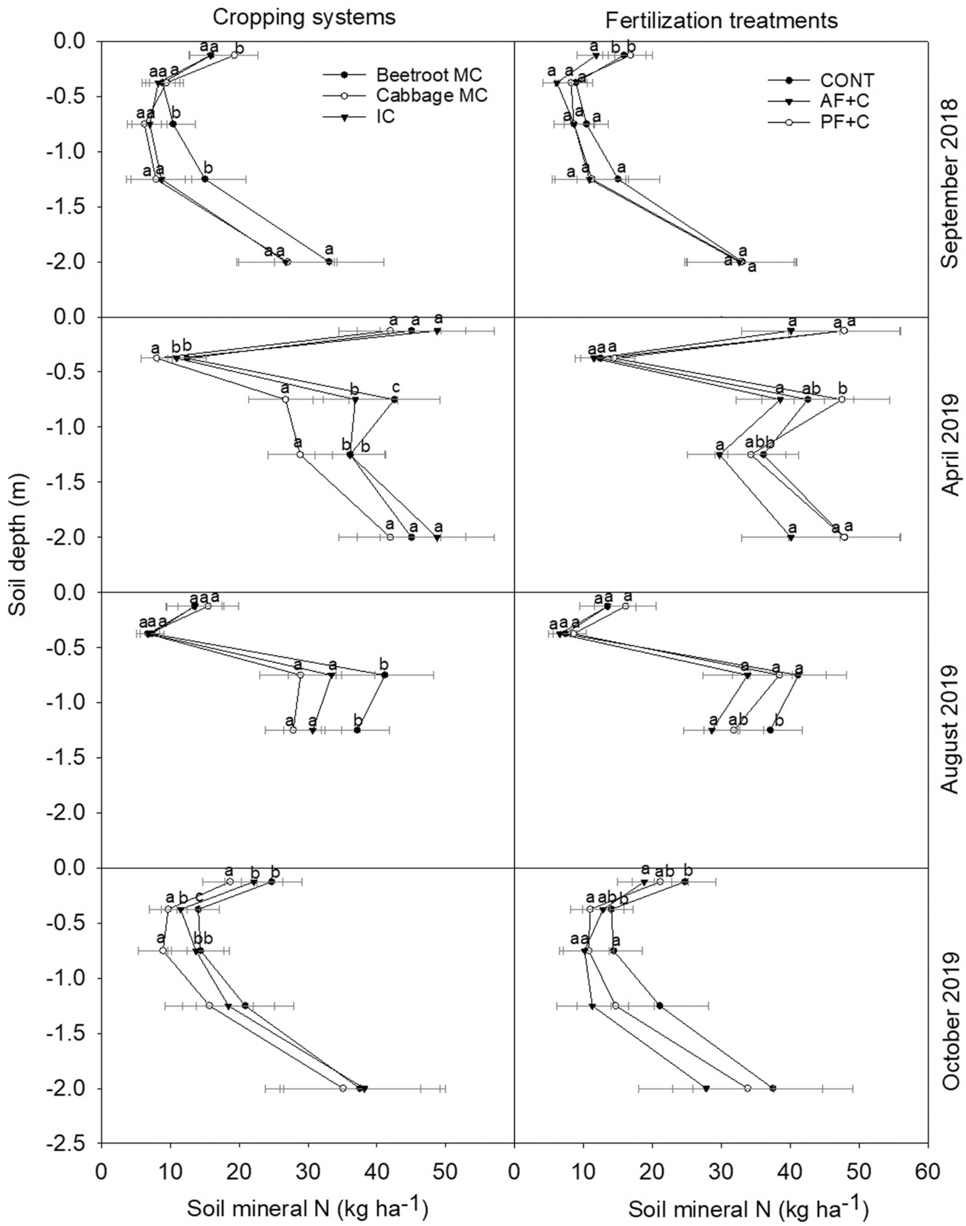

Fig. 4. Soil mineral N in the top soil layer (0–2.5-m depth) in September 2018, April 2019, August 2019, and October 2019. Cropping systems: Cabbage MC, cabbage monocropping; Beetroot MC, beetroot monocropping; and IC, intercropping. Fertilization strategies: CONT, pig slurry; AF+C, animal-based fertilizers and compost; and PF+C, plant-based fertilizers and compost. Bars indicate 95% confidence intervals, n = 4. Different lowercase letters indicate significant differences among treatments ($P < 0.05$). Crop plots were interchanged in 2019 (i.e., cabbage MC plots and beetroot MC plots in 2018 became beetroot MC plots and cabbage MC plots in 2019 respectively. Under IC, crop rows were interchanged.

substitution design (Santos et al., 2002) and with lettuce under field conditions (Stavridou et al., 2012), only one of the crops produced a good yield.

In 2019, the interval from beetroot sowing to cabbage transplanting was extended by 11 days compared to 2018, which likely gave beetroot an advantage in competing in the IC system (Table 1). This supposition is supported by our observation of a 10 % yield increase in beetroot total and marketable yield in the IC system compared to beetroot MC system, calculated per meter row. Management practices, such as altering sowing/transplanting times, can affect the competitive ability of component crops to acquire resources (Xie and Kristensen, 2017). In a beetroot-chicory IC study, simultaneous transplantation resulted in reduced beetroot productivity relative to when chicory was transplanted 7 or 14 days after beetroot sowing (Coutinho et al., 2017). Delaying cabbage transplanting in our study reduced the total and marketable yields (calculated per meter row) of cabbage in an IC system by 28 % and 37 %, respectively, compared to cabbage MC yield values. Overall, in 2019, the productivity of IC systems was comparable to MC systems with a LER of 0.96. Adjustments in management practices can improve yields in IC systems, as evidenced by the increased LER from 0.83 in 2018 to 0.96 in 2019. This increase emerged when cabbage-transplanting was delayed in 2019 compared to 2018. In conclusion, our first hypothesis of increased productivity and land use efficiency under cabbage-beetroot IC was rejected in this study, and the lack of increase was attributed to competition.

4.2. Effects of IC on root growth and N dynamics

The roots of our cabbage and beetroot plants grew to depths of 1.7–2.0 m and 1.8–2.0 m, respectively, which are comparable to depths reported for these crops under organic conditions in previous studies (i.e., 1.7–2.4 m and 1.8–2.1 m, respectively; (Hefner et al., 2019; Kristensen and Thorup-Kristensen, 2007)). Root intensity$_{mod}$ was greater in cabbage MC than in beetroot MC from most depths at most sampling times in 2018 (Fig. 2, Fig. 3). This greater intensity could have given cabbage plants an advantage over beetroot plants in foraging for water and nutrients in our IC system. This difference was confirmed by our observation of a higher partial LER and increased productivity for

Table 7
Comparisons of nitrogen (N) uptake efficiency (NUpE) and N balance across cropping systems and fertilization strategies.

Factor	NUpE (%)		N balance (kg ha^{-1})	
	2018	2019	2018	2019
Cropping system				
Cabbage MC	80^{b} (73–87)	68^{a} (60–77)	123^{a} (101–145)	198^{b} (154–242)
Beetroot MC	72^{a} (65–78)	93^{c} (83–103)	162^{b} (137–188)	121^{a} (87–155)
IC	70^{a} (64–77)	80^{b} (71–90)	139^{a} (115–162)	165^{ab} (126–205)
Fertilization strategy				
CONT	72^{b} (65–78)	93^{b} (83–103)	163^{a} (137–188)	121^{a} (87–155)
AF+C	58^{a} (52–64)	67^{a} (59–76)	185^{ab} (158–211)	195^{b} (153–237)
PF+C	54^{a} (48–60)	68^{a} (59–77)	214^{b} (185–243)	247^{b} (198–295)
P-value				
Cropping system	0.008	< 0.001	0.008	< 0.001
Fertilization strategy	< 0.001	< 0.001	< 0.001	< 0.001
Cropping system × fertilization strategy interaction	n.s.	n.s.	n.s.	n.s.

Estimates are given with 95 % confidence intervals in parenthesis (n = 4). Lower-case letters indicate significant differences among treatments. Abbreviations for treatments are as in Table 6. N.s. = not significant.

Table 8
Potential soil N mineralization (PMN) (mg kg^{-1} 28 days^{-1}) in 0–0.25-m depth soil by fertilization strategy in 2019 at four time points. An interaction between cropping systems and fertilization strategies was found to be significant in August.

	May	June	August			October
			Cabbage MC	Beetroot MC	IC	
CONT	34^{a} (24–45)	53^{a} (42–63)	64^{ab} (58–69)	60^{a} (55–65)	59^{a} (54–64)	55^{a} (50–61)
AF+C	47^{a} (35–59)	58^{a} (48–68)	66^{ab} (60–71)	66^{ab} (60–71)	68^{abc} (63–74)	62^{a} (56–68)
PF+C	40^{a} (29–51)	90^{b} (77–103)	68^{abc} (62–74)	82^{c} (76–89)	75^{bc} (69–81)	59^{a} (53–65)

Estimates are given with 95 % confidence intervals in parenthesis (n = 4). Different lower-case letters indicate significant differences among treatments ($P < 0.05$). Cropping systems: Cabbage MC, cabbage monocropping; Beetroot MC, beetroot monocropping; and IC, intercropping. Fertilization strategies: CONT, pig slurry; AF+C, animal-based fertilizers and compost; and PF+C, plant–based fertilizers and compost.

cabbage, relative to beetroot, within the IC system in 2018. In the IC plots, both cabbage and beetroot rows had neighboring intercrop roots present. However, the presence of cabbage roots under the neighboring beetroot rows were higher compared to the presence of beetroot roots under the neighboring cabbage rows (visual observation). This could explain the significantly higher root intensity$_{mod}$ observed for beetroot IC in some soil layers during August and September of 2018 compared to beetroot MC, while the presence of beetroot roots under cabbage rows were not enough to significantly increase the root intensity$_{mod}$ of cabbage IC.

In July 2019, root intensity$_{mod}$ of beetroot was similar across IC and MC systems. This difference relative to 2018 could be due to the delayed sowing of cabbage in 2019 resulting in less of a competitive disadvantage for space and resources for beetroot roots. Similarly, in a prior greenhouse experiment, (Andersen et al., 2014) found that beetroot roots had pre-empted the soil layers for nutrient resources before clover roots reached them, which lead to decreased performance of clover in an IC system. IC crops with different rooting patterns (deep and shallow) and resource acquisition (legume, non-legume) can result in an

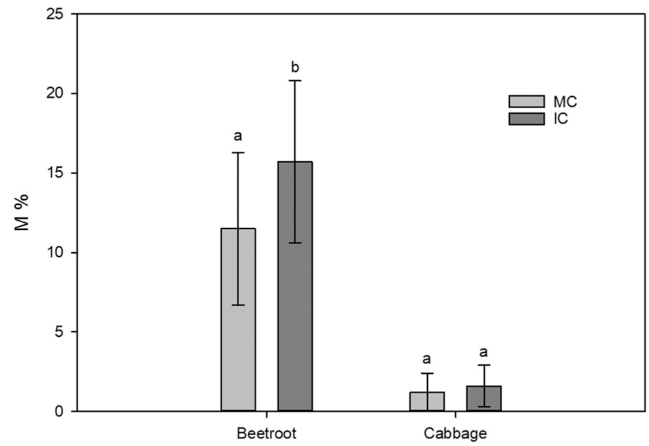

Fig. 5. Mycorrhizal colonization intensity (M%) of beetroot and cabbage roots in 2019. Cropping systems: MC = monocropping, IC = intercropping. Bars indicate 95 % confidence intervals, n = 4. Different letters indicate significant differences among treatments ($P < 0.05$).

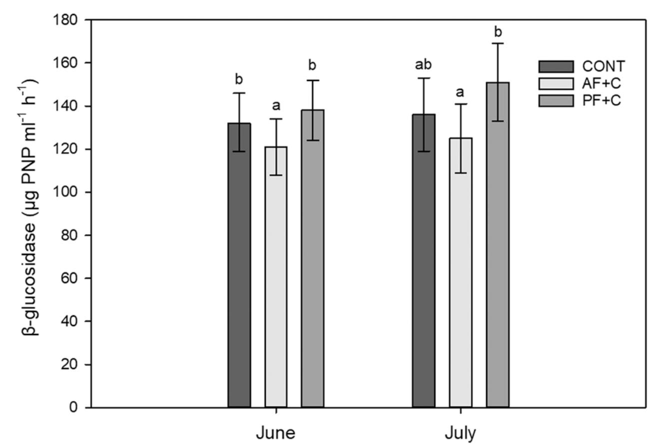

Fig. 6. β-glucosidase activity in 0–0.3-m-deep soil in 2019. Fertilization strategies: CONT, pig slurry; AF+C, animal-based fertilizers and compost; and PF+C, plant-based fertilizers and compost. Bars indicate 95% confidence intervals, n = 4. Different lower-case letters, within each month, indicate significant differences among treatments ($P < 0.05$).

increased NUE through complementation (Shanmugam et al., 2022). However, we did not find any advantage of IC for lowering the N balance or depletion of soil N from deep layers, as evidenced by our results showing that soil mineral N did not differ between cropping systems in deeper layers at harvest. The LER-N data revealed similar N accumulation levels across our IC and MC systems. Thus, our IC system resulted in a competing root interaction rather than a complementing one, showing the lack of spatial, temporal, and resource niche separation in cabbage-beetroot intercropping.

In conclusion, seemingly supporting the first part of our second hypothesis, root intensity$_{mod}$ was enhanced in beetroot IC rows in some soil layers in 2018. However, this enhanced root intensity$_{mod}$ did not result in any yield advantage or increased N uptake from deep soil layers in the IC system in 2018, nor in 2019. Thus, ultimately, our second hypothesis on complementary root growth and nutrient use efficiency of the IC system was rejected.

4.3. Effects of fertilization strategy on crop yield and root growth

The nutrient availability associated with particular fertilization strategies is important for determining crop yield. The higher yield and

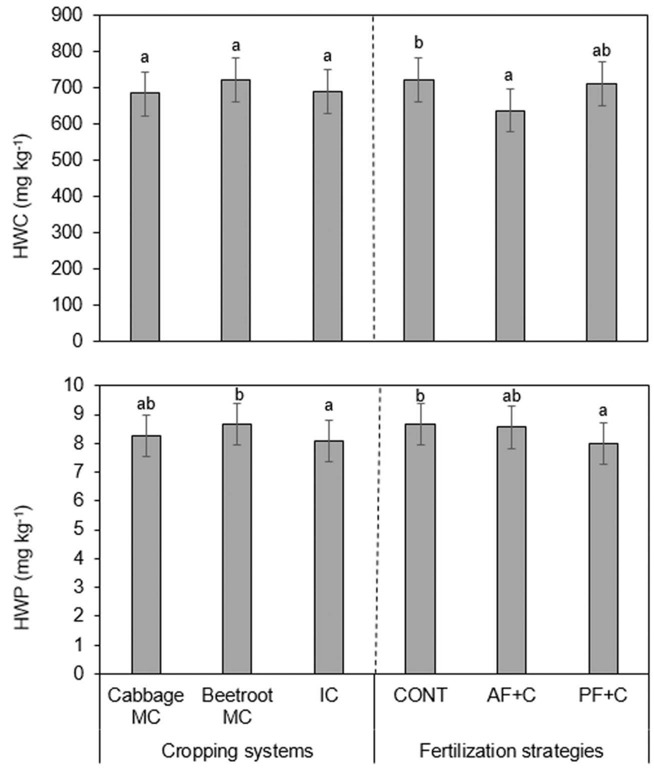

Fig. 7. Hot water extractable carbon (HWC) and phosphorus (HWP) in 0–0.3-m-deep soil in 2019. Cropping systems: Cabbage MC, cabbage monocropping; Beetroot MC, beetroot monocropping; IC, intercropping. Fertilization strategies: CONT, pig slurry; AF+C, animal-based fertilizers and compost; PF+C, plant-based fertilizers and compost. Bars indicate 95% confidence intervals, n = 4. Different lower-case letters indicate significant differences among treatments ($P < 0.05$).

nutrient accumulation that we observed under CONT in 2018 could be consequent to the high nutrient availability in animal manure in the form of mineral N (Ros et al., 2007). In contrast, a lower crop yield with the utilization of fertilization strategies that were designed with both fast and slow N releasing organic amendments (AF+C, PF+C) in 2018, could be due to an overall slower N release compared to that from pig slurry in CONT. Crop yield response to organic amendments is quite limited in the first three years owing to their slow mineralization rate and nutrient release, after which the effect increases gradually (Luo et al., 2018). In our study, the PF+C crops already had similar yields as the CONT crops by 2019 (Table 5). This outcome in 2019 could be related to residual nutrient release from the first-year amendment as well as the higher total N supply and availability under PF+C, compared to CONT, which was also confirmed by higher PMN values in June and August of 2019. Relatively lower crop yields under AF+C in both years could be attributed to recalcitrant C presence in the biogas digestate and deep litter manure (Gebremikael et al., 2020) or a lower N input compared to CONT and PF+C conditions in 2018 and 2019, respectively (Table 4).

The strategy of employing compost together with plant-based fertilizers under (PF+C) was associated with increased rooting intensity$_{mod}$ of beetroot MC (0.5–0.75 m soil layer) in 2018 and cabbage MC (0.75–2 m soil layer) in 2019 compared to the rooting intensity$_{mod}$ obtained with the use of pig slurry without added compost under CONT (Fig. 3). Similarly, Gaiotti et al. (2017) showed that vineyard-pruning compost use resulted in grape plant root growth that was greater than that observed with the use of cow manure compost in terms of total root density m^{-2} down to a 1-m soil depth. The increased root growth extending beyond the local application of compost was attributed to overall increased nutrient availability and crop growth. At the time of harvest in 2018, we observed greater beetroot root intensity$_{mod}$ in the deep soil layers (0.75–2.0 m) of our IC plot supplied with pig slurry (CONT) than in that fertilized with PF+C that could be due to a higher nutrient availability. Similarly, Baldi and Toselli (2013) found that cow manure fertilization tended to benefit deeper root growth at depths in the range of 0.4–0.8 m, whereas compost tended to benefit root growth in the 0.2–0.4-m soil layer. Many factors, such as N status of the soil, the presence of nutrient-rich soil patches, N availability of fertilizers, and type of fertilizer, affect root growth patterns (Baldi and Toselli, 2013; Robinson et al., 1999; Xie et al., 2021). Evidence of fertilizer effects on rooting intensity$_{mod}$ in our study was scant, being limited to significant differences in very few layers, with these rare differences being associated with different cropping systems with no consistent pattern. Our third hypothesis regarding increased root growth under combined application of fast N releasing fertilizer and compost was supported only partially for beetroot MC and cabbage MC systems. Our third hypothesis regarding yield improvement was thus rejected because the yields were either similar or lower under combined fertilization conditions compared to the CONT condition.

4.4. Fertilization strategy effects on N and P pools

Compared to fertilization that included compost (PF+C, AF+C), fertilization with pig slurry (CONT) resulted in a higher NUpE in both study years and a higher plant N accumulation in 2018, which could be due to greater N availability. Although absolute N input was higher for fertilization strategies that included compost (PF+C, AF+C), NUpE in the compost-inclusive conditions was lower than that obtained with pig slurry, perhaps because the N in compost is mostly in the form of organic N which is not easily mineralized (Ciaccia et al., 2017).

Despite the lower NUpE observed with the use of compost, N accumulation under PF+C was similar to that observed with pig slurry in 2019, presumably owing to the increased N input and N availability in the PF+C condition in June and August of 2019, as indicated by our higher PMN data. We can deduce that a higher N balance, indicative of a greater N surplus, with the AF+C and PF+C fertilization strategies compared to that observed in the CONT condition did not result in an increased risk of N leaching because we obtained similar soil mineral N values in deep soil layer samples across the treatments (Fig. 4). Likewise, Canali et al. (2012) found that applying a combination of clover green manure and compost did not increase soil mineral N at the end of the potato cropping cycle compared to treatments without green manure, indicating that higher nutrient input might not increase nitrate leaching risk when the soil supplements are supplied from slow releasing sources. Since the N surplus did not translate into higher N accumulation or soil mineral N, it indicates that in the long term, compost application increases soil organic N, which may result in a higher yield and soil microbial activity (Diacono and Montemurro, 2011), whereas the long term effect on the risk of leaching is not well described except in situations with higher precipitation and a lack of winter cover crop.

The relatively higher P input in animal-based fertilizers was reflected by higher HWP values under both of the animal-based fertilization strategies (CONT, AF+C) compared to the completely plant-based fertilization strategy (PF+C)(Table 3, Fig. 7). Despite the lower P input of the compost and clover fertilizer material in the PF+C, plant P accumulation was similar to that in the CONT in 2019. This could be either due to the average P levels (30 mg kg^{-1}) of trial sites or increased P solubility through humic substances (especially humic acid), which are present in compost and produced through decomposition of plant litter. This effect is caused by the soil pH buffering ability, promotion of soil macroaggregates that can accumulate organic P and reduce P loss through erosion/leaching, increased abundance of P solubilizing microorganisms and phosphatase activity (Yang et al., 2021). Additionally, green manures increase soil microbial and enzyme activities that can increase P availability (Piotrowska-Długosz and Wilczewski, 2020). However, it should be considered that a possible P mining effect together with the lower P contribution from 100 % plant-based sources may deplete soil P reserves, especially from low-P soils, in the long term (Ciaccia et al., 2017). Although the productivity and nutrient availability observed with animal-based and plant-based fertilization were similar in the second year, the long-term risk of P saturation in P-rich soils fertilized with pig slurry and other animal-based fertilizers should also be considered (Guardini et al., 2012). The second part of our third hypothesis regarding increased nutrient availability under conditions of improved organic fertilization was supported only when clover combined with compost (PF+C) was applied, as indicated by increased PMN, with no advantage on nutrient accumulation.

4.5. Plant-based fertilization

Fertilization with a combination of plant residue from fresh clover and compost (PF+C) resulted in crop yields that were similar to those obtained with pig slurry (CONT) in 2019, confirming our fourth hypothesis that plant-based fertilization can achieve similar yields as those achieved with animal-based fertilization. Rothe et al. (2019) found that a one-time application of green manure combined with compost resulted in a yield of pineapple similar to that obtained with conventional fertilization on a split application protocol. They also found that even though the mineralization rate of compost was lower than that of feather and blood meal, the nutrient availability in compost treated soil was enhanced owing to improved soil properties reflected by a higher indicator of potential residual organic carbon. Our study shows that plant-based fertilization strategies can result in similar yields as animal manures and that plant-based fertilization has the additional benefit of reduced P accumulation in soil compared to pig slurry, a property that is particularly useful for preventing P saturation in P-rich soils and can improve recycling of plant-based wastes. In conclusion, the use of plant-based fertilizers can help reduce the use of conventional animal manures in organic vegetable production without compromising yields.

4.6. Effects of fertilizer treatment and IC on soil fertility

The presently examined fertilizer strategies that were inclusive of a slow releasing compost amendment (PF+C, AF+C) did not stimulate indicators of soil fertility by soil enzyme activity beyond the stimulation produced by pig slurry (CONT). β-glucosidase catalyzes the final step of cellulose degradation to produce glucose, which is an important energy source for microbes, and compost addition increased β-glucosidase activity in soil supplied with organic matter (Adetunji et al., 2017). In our study, higher β-glucosidase activity was found under CONT and PF+C than AF+C. This difference is likely consequent to the high amount of recalcitrant C that is present in biogas digestate and deep litter manure (AF+C) (Gebremikael et al., 2020). The presently observed high enzyme activity in soil fertilized with pig slurry (CONT) can be explained by the predominant presence of labile organic C in pig slurry, which increases enzyme activity (Yanardağ et al., 2020), though the total C added via pig slurry accounted for only 6% and 8% of the total C added in the PF+C and AF+C fertilizers, respectively, in 2019 (Table 4).

Dehydrogenase is an intracellular enzyme in soil microorganisms that can be used as an indicator of overall microbial activity in soils (Adetunji et al., 2017). We found that dehydrogenase activity and microbial biomass C, N, and P levels were similar among the fertilizer strategies at the end of the second year of our study. Less organic C was added in CONT than was added with other two treatments, but all three fertilizer strategies included biomass from incorporated winter cover crops, which might have overshadowed differential effects of the fertilizer strategies. However, increased microbial activity under PF+C supplemented soils in June 2019 was evidenced by a higher PMN (Table 8). Subsequently, in August, we found an interactive effect among cropping systems and fertilizer strategies, wherein all cropping systems except cabbage MC, maintained with PF+C fertilizer had a higher PMN than the same systems maintained with AF+C or CONT fertilizers. Miltner et al. (2012) found that 40 % of the added microbial biomass ended up as a part of soil organic matter in an incubation experiment, indicating the importance of microbial activity in improving soil fertility.

Beyond short-term benefits, potential advantages of compost addition in an organic fertilization strategy should be considered with a long-term perspective. In the long-term, use of compost increased soil organic matter levels and improved soil quality (Diacono and Montemurro, 2011). Higher dehydrogenase activity has been found in compost-treated soils, compared the manure-treated soils, in only the second year of use (Ros et al., 2007). IC in the present study had a positive effect on the mycorrhizal colonization of beetroot that was similar to the increased mycorrhizal colonization observed previously for intercropped maize roots grown with companion crops such as squash, beans, and vetch combined with organic inputs (Mariela et al., 2016). Mycorrhizal fungi are known to improve soil fertility by increasing P availability to plants, reducing soil-borne fungal pathogens (e.g., *Phytophthora*, *Fusarium*, *verticillium* etc.), and promoting water-stable soil macroaggregates (Jeffries et al., 2003). Even though, IC increased microbial colonization, an interactive effect of fertilizer strategies and cropping systems in improving the soil fertility was evidenced only in our PMN data, consistent with hypothesis 5.

5. Conclusion

Advantages of IC for system productivity and NUE were not found due to competition between cabbage and beetroot and a lack of complementary niche separation in terms of root growth and N use. However, displacement of sowing dates in the second year reduced the level of competition. In this year, IC increased the mycorrhizal colonization of beetroot roots compared to MC where beetroot was the stronger competitor in IC due to early sowing compared to cabbage. Improved organic fertilization strategy that included a fast N releasing fertilizer and a slow N releasing compost did not increase yield, NUE, or soil enzyme activity compared to pig slurry. However, PMN was found to be higher under plant-based fertilization with compost. Although benefits of compost addition were not seen in the short term of 2 years, a longer-term positive effect on yield and improved soil fertility is possible owing to cumulative effects of organic C and N inputs. Plant-based fertilizers can be an efficient alternative to animal-based fertilizers without compromising yield and with similar P accumulation in crop plants, especially where conventional animal manures are used in organic production. Cropping systems and fertilization strategies did not interact in affecting crop yield and soil fertility except for PMN. Investigations on potentially complementing crop species and fertilization strategies should be continued to increase the productivity and nutrient use efficiency of organic vegetable production in a sustainable way.

CRediT authorship contribution statement

Sindhuja Shanmugam: Conceptualization, Formal analysis, Investigation, Writing - Original Draft, Writing - Review & Editing, Visualization. **Margita Hefner:** Investigation, Writing - Review & Editing, Visualization. **Rodrigo Labouriau:** Methodology, Formal analysis, Writing - Review & Editing. **Alessandra Trinchera:** Conceptualization, Methodology, Formal analysis, Investigation, Writing - Review & Editing, Funding acquisition. **Koen Willekens**: Conceptualization, Methodology, Investigation, Writing - Review & Editing, Funding acquisition. **Hanne Lakkenborg Kristensen:** Conceptualization, Methodology, Investigation, Writing - Review & Editing, Visualization, Funding acquisition.

Declaration of Competing Interest

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

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