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SHORT-TERM EFFECTS OF BRASSICA COVER CROPS ON SOIL QUALITY INDICATORS IN ORGANIC PRODUCTION IN HIGH TUNNELS

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ABSTRACT

The use of cover crops is aimed at sustaining soil health and productivity in the context of agricultural intensification and accompanying soil degradation. While cover crops have been extensively studied in field production systems, limited research has been conducted concerning their application in high-tunnel vegetable production. This study aimed to assess the effects of turnip (*Brassica napus* subsp. *napobrassica* (L.) Jafri) and swede *Brassica rapa subsp. Rapifera* Metzg) cover crops (CCs) on soil physicochemical and biological properties in an organic high tunnel system in southern Poland in 2017–2019. The planting sequence was as follows: winter cover crops/pumpkin/romaine lettuce/broad bean/chilli pepper. Soil analyses included measurements of bulk density, water capacity, soil aggregation, soil organic carbon (SOC), available soil nutrients, as well as microbial abundance and diversity. Rape crops produced a higher aboveground dry biomass (4.11 t ha^{-1}) than swede (2.85 t ha^{-1}), and the N content in their biomass was 101 kg N ha^{-1} and 75 kg N ha^{-1}, respectively. The results presented that CC residue significantly contributed to soil organic carbon stock, retention of plant-available nitrogen, and improvement of soil physical properties, especially wet aggregate stability. Soils with the highest SOC concentrations were associated with the highest bacterial and fungal abundance. The most significant number of mesophilic bacteria was detected in the soil where turnip was grown as a cover crop (7.6 × 10^{7} cfu g^{-1} DM soil). Moreover, a higher abundance of the tested nitrogen cycle bacteria was found in the soils after CC cultivation compared to the control soils, particularly bacteria reducing NH_4^+-N and NO_3^--N. These findings highlight the importance of cover crop management practices in high tunnels, as they influence the composition of the total bacterial community and the abundance of N-cycling microbial guilds.

Key words: swede (rutabaga), *Brassica napus* subsp. *napobrassica* (L.), turnip, *Brassica rapa* subsp. *rapifera* Metzg., soil organic carbon, nitrogen cycle bacteria, fungi

INTRODUCTION

Sustainable agriculture encompasses various strategies for effective plant and soil management. One such strategy is cover cropping, which offers several benefits to agroecosystems, including enhanced soil quality, increased nutrient cycling, and improved plant growth and yield [Thorup-Kristensen et al. 2003, Robacer et al. 2016, Adetunjia et al. 2020, Magdoff and Van Es 2021, Scavo et al. 2022]. Incorporating co-

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ver crop biomass helps maintain soil organic matter, leading to better water retention and nutrient availability for crops. The activity of microorganisms and macrofauna influences soil structure, soil particle distribution and decomposition of organic residues. However, the successful sustainable cultivation of vegetables using these practices remains a challenge in terms of agricultural production [Thorup-Kristensen et al. 2012, Larkin 2020].

Intensive vegetable production in high tunnels, with multiple crops grown annually, high temperatures, and protected irrigation, requires inputs of organic materials to preserve soil organic matter and maintain soil quality, thereby ensuring long-term sustainable productivity. Soil degradation is a significant issue for organic producers, who strive to maintain or improve the physical, chemical, and biological condition of the soil. The practice of cover cropping could potentially serve as a management tool to address challenges associated with intensive high tunnel production. Although cover crops have been extensively described in terms of field production, research related to their use in vegetable production in high tunnels is limited [Domagała-Świątkiewicz et al. 2019, Domagała-Świątkiewicz and Siwek 2022, Perkus et al. 2022].

Many plant species belonging to different families can be adapted as cover crops. Plants from the family Fabaceae and grasses are commonly applied, but there is also an increasing interest in brassicas [Magdoff and Van Es 2021]. Members of the family *Brassicaceae*, including yellow and white mustard (e.g., *Sinapis alba* L.), oilseed rape (*Brassica napus* L.), radish (*Raphanus sativus*) and winter oilseed rape (*Brassica napus* L.) are increasingly utilised as cover crops in temperate regions [Gieske et al. 2016]. These species can be grown as short-season cover crops or, in suitable climates, as overwintering cover crops [Thorup-Kristensen et al. 2003, Haramoto and Gallandt 2004]. A combination of a large dominant tap root with a high density of fine active roots characterises brassica cover crops (CCs). This attribute appears to make certain brassica species highly valuable in cover cropping strategies, particularly in terms of nitrogen scavenging. Their residues rapidly decompose, releasing nutrients, and have the potential to improve soil physical properties such as structure and aggregation [Saleem et al. 2020, Koudahe et al. 2022].

The interaction between higher plants and soil microflora, whether in natural or agricultural communities, plays a crucial role in shaping soil properties. Their cooperation encompasses a wide range of soil and microbiological processes, including nitrogen transformations [Grzyb et al. 2021]. Soil microorganisms are susceptible to soil type, temperature, moisture and soil acidity and also depend on agricultural management practices. Microbial communities living in different environments have access to resources with varying chemical compositions and nutrient availability [Mooshammer et al. 2014]. The benefits of cover cropping for soil microbial ties have been attributed to the input of labile carbon from above- and below-ground biomass production [Adetunjia et al. 2020].

An increase in the relative amount of N during the decomposition of organic residues is a common and well-known phenomenon [Thorup-Kristensen et al. 2003, Mooshammer et al. 2014]. Grzyb et al. [2021] reported that the amount of N entering the soil as a result of crop residue mineralisation ranges from 15 to 45 kg N ha^{-1} for cereal residues and from 80 to 144 kg N ha^{-1} for winter oilseed rape residues. Initially, N in organic debris is limited, so decomposer microorganisms utilise most of the N stored in the bodies of soil organisms, preventing nutrient loss through leaching. Consequently, most of N is retained while the C content progressively decreases, leading to a lower C : N ratio as decomposition proceeds. When organic residues with a high C : N ratio (<25 to 30 : 1, N content < 1.4–1,8%) are incorporated into agricultural soils, net immobilisation of N ally occurs [Brennan and Acosta-Martinez 2017, Sarkar et al. 2020]. Legume residues have a low C-to-N ratio, which accelerates net mineralisation and nitrification after their incorporation into the soil [Mooshammer et al. 2014]. Residual brassica CCs, with their low C : N ratio, can also decompose rapidly and provide a significant amount of plant-available nutrients in the process, similar to legumes [Dean and Weil 2009, Koudahi et al. 2022].

Further research efforts are required to regulate the use of cover crops under different cropping systems and varying climatic conditions. Long-term studies are particularly needed to assess the effects of cover crops on soil properties, quality, and subsequent crop yields [Koudahe et al. 2022]. At present, some studies have demonstrated the suitability of cover crops in agricultural production, many of which focused on biological properties; however, there are still only a few studies that investigated the use of such solutions in unheated plastic tunnels. Considering the premium value of space in high tunnel systems, soil quality becomes critically important.

The purpose of our research was to quantify the effect of brassica winter cover crops in high tunnels on various soil quality indicators, including their physical and chemical parameters. We also focused on understanding the effects of cover crop practices on microbial abundance and diversity, particularly the bacterial communities involved in nitrogen-cycling processes. The experiment involved a triple-cropping system in the first year, where three crops were planted and harvested in the same area, including the cover crop, followed by a twice-cropped system in the second year. Our study specifically investigated the changes in soil quality that were influenced by the specific crop and the short-term dynamics during the intensive crop rotation with cover crops in a cold high tunnel system.

MATERIAL AND METHOD

Site, location and climate. In Poland, the winter months between October and March provide an ideal opportunity for winter cover cropping without interfering with the routine income-generating rotations. Non-leguminous, broadleaf turnip (*Brassica rapa* subsp. *rapifera* Metzg) and swede, i.e., rutabaga (*Brassica napus* subsp. *napobrassica* (L.) Jafri.) were used as cover crops in high tunnel organic production. Swede and turnip plants are known for their cold hardiness (–6°C) and drought tolerance. The microclimatic conditions in the STN 070 high plastic tunnel, including photosynthetic active radiation (PAR), air temperature, relative air humidity, and air CO2 content, were extensively described in a separate paper [Siwek et al. 2020]. These conditions were significantly influenced by the weather, with more significant variations observed on cloudy days, particularly in the hours before and after midday. No significant differences were observed between tunnel zones for individual parameters, especially on a sunny day.

Experimental design and agronomic management. The experiment was established in 2017 at the experimental station of the University of Agriculture in Kraków in Mydlniki, Poland. This region in southern Poland (51°13'N, 22°38'E) has a humid continental (Dfb) climate according to Köppen's classification [Peel et al. 2007]. A tunnel (30 m long, 7 m wide and 3.2 m high) covered with LDPE (low-density polyethylene; 0.165 mm thick) film was built in an east-to-west orientation at the site in March 2014. According to PN-R-04032:1998, particle size analysis categorised the soil at the site as fine-textured, matching the silty clay soil group, with approximately 40% of clay particles. The soil fertility management used in this trial included 20 t ha^{-1} cow manure in 2014, supplemented with potassium sulphate 50% K_2O and 44% SO_3 (500 kg ha^{-1}) in the autumn of 2018 after the romaine lettuce harvest. All amendments were incorporated into the soil in autumn to a depth of 15 cm. The cultivation plots were established in the high tunnel in the spring of 2017 in four replicates, with each plot measuring 15 m^2. Before the experiment, the soil was prepared using a mechanical rotary cultivator. From October 20, 2017, to April 24, 2018, the turnip (*Brassica rapa* subsp. *rapifera Metzg*) cultivar 'Rogowska' and swede (rutabaga, white turnip; *Brassica napus* L. var. *napobrassica*) cultivar 'Saba' were planted as cover crop treatments, without fertiliser application. The seeding rate for cover crops was 20 kg ha^{-1}. Seeds were sown manually onto the plots and covered with soil using shallow cultivation methods.

Table 1 presents the operations conducted in the high tunnel from 2017 through 2019. In early spring, on April 24, 2018, the biomass of cover crops (CCs) was incorporated into the top 10 cm layer of soil as green manure.

Moving and incorporation were considered as termination of cover crop cultivation. The control treatment was bare soil protected with black plastic mulch until planting the main crop.

'Uchiki Kuri' (HILD) seedlings were planted in the high tunnel on May 25, with a row and plant spacing of 150 × 100 cm. The average daily temperature outside the tunnel in April was 8.9°C, and the minimum temperature was 3.7°C. The pumpkin crop started yielding on July 16 and continued until August 25, 2018. The plots were irrigated using dripper lines. Weed removal was necessary during the early stage of pumpkin growth. After the pumpkin harvest, roma-

Table 1. High tunnel operations in vegetable production with cover crops (2017–2019)

Year	Date	Operation
2017	October 20	sowing cover crop
2018	April 24	**cover crop termination**, collecting soil samples for analysis, soil cultivation
	April 1	planting of **pumpkins** in multiplates (glasshouse)
	May 25	planting of pumpkin seedlings
	July 16–August 25	pumpkin harvest, collecting soil samples for analysis
		planting of **romaine lettuce** in multiplates
	July 2	
	August 30	planting of romaine lettuce seedlings
	October 3	romaine lettuce harvest
	November 22	soil mulching of sorghum straw
2019	April 6	planting of **broad bean**
	May 9	collecting broad bean leaves for analysis, collecting soil samples for analysis
	June 19	harvest of bread bean
	June 24	planting of **chilli pepper**
	August 19–October 22	harvest of chilli pepper, collecting soil samples for analysis

ine lettuce (*Lactuca sativa* var. Romana, var. Xavira) (HILD) was planted on August 30, 2018, with a spacing of 30 × 20 cm. Harvesting of romaine lettuce was completed on October 3. Following the lettuce harvest, the plots were mulched with sorghum straw to a depth of 10 cm.

In the spring of 2019, broad beans (*Vicia faba*) were sown without any tillage at a row and plant spacing of 50 × 10 cm. The plants were harvested on June 19. Chilli pepper was the last plant species in the two-year crop rotation cycle with cover crops in the experimental high tunnel. Chilli pepper seedlings were transplanted to the tunnel on June 24 at a spacing of 100 × 100 cm. The fruits were harvested between August 19 and October 22. There were five harvests in total, with the first four harvests at full maturity by the end of September and the last harvest at technical maturity (5 times in full maturity by the end of September and the last time in technical maturity). The production in the high tunnel followed the standards of organic farming, as indicated by the organic farming certificates (Pl-03-02786-16; PL-03-002786-17).

Soil sampling and analyses. Soil sampling was conducted using a soil core sampler after the end of cover cropping (April 24, 2018) and subsequently after each cash crop harvest from a depth of 0–20 cm of soil. For bulk density measurements, undisturbed soil samples were randomly collected in four replicates separately from each treatment, and each replicate from a depth of 0–10 cm was collected using a 250 cm^3 Kopecký's cylinder. A total of 36 intact soil samples were collected. The soil cores were weighed, wetted to facilitate capillary action, and then dried at 105°C. Aggregates of intact bulk soil at a depth of 0–20 cm were collected from each plot with six replicates – air-dried aggregates (<5 mm) were obtained by sieving bulk soil. Briefly, 40 g of each soil sample was placed on the uppermost sieve of a nest of five sieves with a mesh size of 0.25, 0.5, 1.0, 1.5, and 2.5 mm and pre-soaked in distilled water for 5 min. Aggregates were wet-sieved using a motor-driven holder, lowering and raising the sieves in a water container with a stroke length of 5 cm. The sieving frequency was five cycles cm^{-1} for 20 min. After wet-sieving, the water-stable soil materials from each sieve were dried in an oven at 105°C, and the proportion of aggregates in each sieve represented water-stable aggregates for the following size classes: 5.0–2.5, 2.5–1.5, 1.5–1.0, 1.0–0.50, and 0.50–0.25 mm. The water-stability aggregate index (WSI) was calculated by summing the fractions of water-stable aggregates in the size range of 0.25–5.0 mm. Soil pH (pH) was determined by mixing soil with distilled water at a ratio of 1 : 2 and measuring the pH of the resulting suspension. Soil organic carbon (SOC) content was determined using the dichromate wet oxidation method based on the Walkley and Black protocol [Ostrowska et al. 1991]. The soluble form of macroelements and sodium was determined by the universal method in 0.03 mol dm^{-3} CH_3COOH. The content of the available form of boron was determined in 1 mol dm^{-3} HCl extractant [Ostrowska et al. 1991]. Nutrient elements were determined using the ICP-OES (inductively coupled argon plasma atomic emission spectroscopy) technique.

Cover crop plant analysis. Before terminating the brassica cover crops, aboveground plant biomass was sampled for analysis from a 4 × 1 m^2 area for each combination. The plants were weighed to estimate the total aboveground fresh biomass.

Aboveground biomass of CCs was randomly sampled during swede and turnip cover crop termination. Additionally, samples of mature leaves from broad bean plants were collected during plant flowering. The collected plant material was washed with distilled water, dried at 65°C for 48 h and then ground. For elemental analysis, wet microwave digestion in HNO3 was applied. Macro- and microelements were determined by the ICP-OES technique using a Prodigy High Dispersion ICP-OES Spectrometer (Teledyne Leeman Labs, Hudson, NH, USA). Quality control of plant material elemental analysis by the ICP-OES method involved measuring the levels of respective elements in the certified material (CRM INCT-PVTL-6, Polish Virginia Tobacco Leaves, Institute of Nuclear Chemistry and Technology). The total nitrogen content in plant biomass was determined using the Kjeldahl method [Ostrowska et al. 1991]. The dry matter content of the cover crop biomass was determined using the gravimetric method PN-A-75101-03:1990.

Microbiological analysis. Quantifying the abundance of soil bacteria is essential to better understand bacterial population dynamics and main soil biogeochemical processes [Schloter et al. 2018, Lee et al. 2021]. The soil microbiome is known to exhibit high temporal and spatial variability, leading to the concept of "hot spots" and "hot moments" in soil [Schloter et al. 2018, Romdhane et al. 2019, Han et al. 2020], where microbial activity and biomass can vary significantly. It is well established that microbial activity and biomass increase rapidly following the introduction of aboveground organic biomass of green manures into the soil. In the present study, soil microbiological analyses were carried out 12 and 19 months after CC incorporation into the soil to demonstrate the subsequent effect of biomass supply on soil microbial activity in intensive vegetable rotations in a high, unheated tunnel. Geisseler et al. [2010] and Grzyb et al. [2021] argued that mineralisation, immobilisation and turnover of nitrogen from plant residues have a long-term beneficial effect on soil productivity and plant nutrition.

For the current study, traditional bioindicators were selected for analysis using culture-dependent methods such as colony forming unit (CFU), spotting, and most probable number (MPN). These indicators characterise organisms that perform essential functions in the agroecosystem with sufficient accuracy. Soil samples were collected in 2019 during broad bean blooming and after the chilli pepper harvest. Soil from each treatment in three replicates was sampled randomly from the 0–20 cm depth. The collected soil samples were kept at 4°C until determination of the total bacterial count, ammonium-oxidising bacteria, nitrite-oxidising bacteria, and denitrifying bacteria, as well as the total population of fungi.

To determine the microbial populations, 10 grams of each soil sample was added to 0.85% NaCl solution. After homogenisation for 30 min, the solution was diluted in serial decimal dilutions (10^{-1} to 10^{-7}), and aliquots of the resulting solutions were plated on appropriate culture media. All media were autoclaved at 121°C for 15 min before use.The content of bacteria and fungi was assessed in water soil extracts using the Koch surface-plating method.

In this study, the most probable number (MPN) technique was used to estimate the counts of ammonifiers, nitrifiers, and denitrifiers in the soil samples. The MPN method involves a series of 10-fold dilutions of the soil samples. Each sample was used to inoculate five tubes for each dilution level in a unique medium. Ammonifiers were incubated at 25°C for seven days. The presence or absence of ammonium was determined using the Nessler reagent.

For detecting nitrite-oxidising bacteria (NOB), Vinogradsky medium was used. Tubes were incubated for 14 days at 30°C. To identify positive samples, the tubes were visually inspected at two-day intervals for

Table 2. Total aboveground biomass and concentration of nutrients in dry biomass of cover crops grown in the high tunnel

Treatment	Fresh biomass (t ha^{-1})	Dry biomass (t ha^{-1})	Dry matter (%)	Total N accumulation (kg N ha^{-1})		
Brassica napus L. var. *napobrassica*	24.6 ±*4.11** a	2.85 a	11.6 ±*0.49* a	75		
Brassica rapa subsp. *rapa*	34.5 ±*3.67* b	4.11 b	11.9 ±*0.38* a	101		
	Macronutrients (% DM)					
	N	Ca	K	Mg	P	S
Brassica napus L. var. *napobrassica*	2.63 ±*0.10** a	1.67 ±*0.09* a	3.86 ±*0.12* a	0.24 ±*0.01* a	0.46 ±*0.02* a	0.71 ±*0.01* b
Brassica rapa subsp. *rapa*	2.45 ±*0.11* a	1.75 ±*0.12* a	4.19 ±*0.17* b	0.22 ±.,*03* a	0.43 ±*0.03* a	0.57 ±*0.06* a
	Micronutrients (mg kg^{-1} DM)					
	B	Cu	Fe	Mn	Mo	Zn
Brassica napus L. var. *napobrassica*	10.6 ±.,*73*	5.1 ±*0.54*	122 ±*14.7*	22.3 ±*1.63*	0.50 ±*0.04*	30.9 ±*4.67*
Brassica rapa subsp. *rapa*	19.4 ±*3.88*	4.4 ±*0.38*	127 ±*16.7*	18.3 ±*3.53*	0.6 6 ±.,*04*	32.7 ±*3.09*

*standard deviation

the production of a sufficient amount of acid to lower the pH and thus change the colour of the marker added as an indicator of nitrifier growth. Nitrite and nitrate were assayed using a standard chemical protocol (Griess-Ilosvay reagent) [Elbanna et al. 2012, Lee et al. 2021].

Giltay medium was used for the cultures of denitrifiers. After ten days of incubation at 30°C, one millilitre of each culture was transferred to a micro-centrifuge tube and analysed for denitrification potential using the alpha-naphthylamine method described by Wistreich and Lechtman [1988].

Statistical analysis. Statistical analyses were conducted to compare the main effects of soil treatments (cover crops) on the variables of interest. The data obtained from soil and biomass analysis were analysed using a one-way analysis of variance (ANOVA). Mean separation was performed using Fisher's LSD test at a significance level of p = 0.05. Data were analysed using Statistica 13.3 software (Statsoft Inc., Poland).

Each microbiological analysis was performed in duplicate, using three independent series of dilutions and culture incubations. The assumptions of variance analysis were verified, i.e. data were checked for normality (Kolmogorov-Smirnov test) and the equality of variance (Levene test). The results of the microorganism counts were analysed using a two-way analysis of variance (ANOVA). The two factors analysed were bacterial counts and cover plants. The significance of differences between the means was assessed using the Duncan test at a significance level of p = 0.05.

RESULTS

Brassica cover crops, whether planted in the spring or fall, can rapidly produce biomass [Magdoff and Van Es 2021]. Oilseed rape cover crops planted in the autumn can yield 3–8 t dry biomass per ha^{-1}, whereas those planted in spring produce 3–4.5 t dry biomass per ha^{-1} [Dean and Weil 2009, Gieske et al. 2016]. In the present study, swede and turnip were grown in an unheated tunnel in the period from October 20, 2017, to April 24, 2018. Turnip produced higher aboveground biomass (4.11 t DM ha^{-1}) than swede (2.85 t DM ha^{-1}), and the N content in their biomass was 101 kg N ha^{-1} and 75 kg N ha^{-1}, respectively. Swede biomass contained higher levels of N, S, Cu and Mn, while turnip plants contained more Ca, K, and B (Tab. 2).

Soil physical and chemical analysis. Winter brassica cover crops significantly increased soil water holding capacity, soil organic carbon (SOC) content and improved soil aggregation compared to the control (Tab. 4). Soil bulk density during the entire rotation period was lower in the soil under swede than in the control soil. Soil analyses performed after the pumpkin harvest (5 months after CC termination) showed the highest content of organic carbon in the soil on the plot with turnips. Considering the crop rotation applied in 2018–2019 (CCs–pumpkin–romaine lettuce–broad bean–chilli pepper), cover crops generally increased the amount of SOC in the soil (Tab. 3). The highest water-stable aggregate index (WSI) was determined in the soils under the swede and turnip cultivation (Tab. 3). The results were similar in both years. The water-resistant soil structure under brassica CCs was characterised by a higher number of aggregates (0.25–0.50 mm in diameter) in both years of organic vegetable production (data not shown).

Cover crop treatments did not significantly affect soil chemical properties after cover crop termination (April 24, 2018) and any of the soil sampling time points (Tab. 4). However, there were some significant differences observed between treatments, primarily concerning mineral nitrogen and sulphates.

Not surprisingly, directly after the termination of cover crops in April 2018, the highest concentration of NO_3-N was recorded for soil from the control treatment, covered with black plastic mulch during the period of CC cultivation (Tab. 4). This is because the cover crops initially reduce nitrogen (N) availability by taking up nitrate and ammonium from the soil. However, as the cover crop residue decomposes, the N becomes gradually available. Elevated NO_3- levels were detected in the soil under the swede cultivation. This plot was also characterised by the highest EC and extractable Na. Five months after the end of CC cultivation in August 2018 (the end of pumpkin harvest), the highest amount of NO_3-N was determined in the cover crop soils. During this time, the predominant form of mineral nitrogen in the soil was ammonium. Additionally, the soil sown with swede also showed the highest content of mineral forms of sulphur (SO_4-S).

Soil analyses conducted in April before sowing broad bean seeds in 2019 and one year after swede and turnip cultivation showed a high content of mineral nitrogen in the form of nitrate. This increase in mineral nitrogen concentration could be attributed to the intensive mineralisation of sorghum straw, which was spread across the entire surface of the tunnel in autumn 2018 for weed protection. In Poland's climate, sorghum does not reach the generative growth stage, and plants remain green for a long time (narrow C : N ratio). Sorghum plants are rich in nutrient elements, crude protein and reducing sugars.

In September 2019, after the harvest of another rotation crop plant, i.e. chilli peppers (18 months after CCs), the highest amount of NO_3-N was detected on the plot after swede cultivation. This soil also contained the highest levels of soluble potassium and sulphur compared to the control treatment (Tab. 4).

After cover crop cultivation (April 24, 2018), the highest amount of sulphates was determined in the soil on the control plots; however, the soil showed an elevated SO_4-S concentration five months after the swede CC treatment (Tab. 4). This pattern was also observed after the chilli pepper harvest on October 22, 2019. In agricultural soils, the relative abundance of sulphates in the soil solution is regulated by the presence of organic matter [Barker and Pilbeam 2017].

Broad bean plant analysis. Crop residues are a valuable natural material that can be used to control many soil physio-chemical parameters and biological functions [Sarkar et al. 2020]. Although broad beans were not cultivated explicitly for green manure, the biological parameters of this plant's growth during flowering are provided in Table 5. The entire aboveground biomass of broad beans formed during vegetation (except for pods) was left as mulch in the tunnel.

Under favourable cultivation conditions, dry broad bean organic matter on an area of 1 ha in the form of post-harvest residues may reach 4–5 t and contain 90–120 kg of bound N. Grzyb et al. [2021] demonstrated that biological N-fixation can increase the amount of nitrogen in soil by 30–50 kg ha^{-1} year^{-1}. Broad beans have a well-developed tap root system that can penetrate the soil to a depth of up to 110 cm, leading to improved physical and chemical properties of the soil. The lateral roots are well-developed and almost as long as the primary root. The coexistence of broad bean with rhizobia (*Rhizobium leguminasarum*) and nitrogen assimilation occurs most intensively at the beginning of budding. The highest number of nodules is found during this period (Fig. 1).

Table 3. Physical soil properties and organic carbon content in soils in the high tunnel in 2018 (after cover crops, after pumpkin), and 2019 (before broad bean planting, and after chilli pepper harvest); n = 2

Treatment	Bulk density	WC		SOC	WSI
	(g cm^{-3})	(%ww)	(%wv)	(%)	(%)
2017	1.29	33.4	42.8	1.27	92.5
Cover crop termination on April 24, 2018					
Control*	1.28 b	32.0 a	40.9 a	1.28 a	90.0 a
Swede	1.16 a	39.8 b	46.0 b	1.40 b	92.3 b
Turnip	1.26 b	36.1 ab	44.4 b	1.32 ab	91.9 b
Pumpkin harvest on August 25, 2018					
Control	1.29 b	35.5 b	46.4 b	1.36 a	88.5 a
Swede	1.24 a	38.6 c	46.6 b	1.37 a	**92.5** c
Turnip	1.35 b	32.2 a	43.6 a	1.42 b	90.6 b
Broad bean blooming** on April 1, 2019					
Control	1.41 b	31.1 a	43.2 a	1.26 a	90.1 a
Swede	1.35 a	32.1 a	45.5 b	1.39 b	90.5 a
Turnip	1.37 ab	30.2 a	41.8 a	1.46 c	90.1 a
Chilli pepper harvest on October 22, 2019					
Control	1.27 a	36.3 a	45.0 a	1.27 a	90.9 a
Swede	1.31 a	32.8 a	43.2 a	1.34 a	95.2 b
Turnip	1.32 a	33.2 a	44.4 a	1.34 a	94.1 b

* control plot in this experiment was bare soil covered with black plastic mulch until the start of the experiment

** broad bean was planted on mulch of sorghum straw; means followed by the same letter in columns do not differ in the Fisher test for $p < 0.05$; water capacity (WC), soil organic carbon (SOC), and water stability index (WSI)

Table 4. Chemical properties of the soil in the high tunnel in 2018 (post cover crop termination, after pumpkin harvest), and in 2019 (before broad bean planting, and after chilli pepper harvest); n = 2

Treatment	pH	EC	N-NH$_4$	N-NO$_3$	P	K	Mg	Ca	S	Na
	(in H$_2$O)	(µS cm^{-1})	(mg dm^{-3} fresh soil)							
2017	7.71	0.09	6.78	2.73	37	85	129	1614	35	21
Cover crop termination on April 24, 2018										
Control*	7.57 a	343 b	0.79 ab	9.60 c	30 a	93 a	133 a	1803 a	48 b	27 ab
Swede	7.40 a	521 b	1.05 b	0.99 a	29 a	75 a	123 a	1401 a	22 a	38 b
Turnip	7.51 a	166 a	0.47 a	4.53 b	34 a	75 a	139 a	1608 a	29 a	21 a
Pumpkin harvest on August 25, 2018										
Control	7.37 a	322 a	3.69 a	1.16 a	34 a	138 a	126 a	1109 a	64 a	28 a
Swede	7.41 a	456 a	1.66 a	2.50 b	42 a	219 a	140 a	1310 a	123 b	35 a
Turnip	7.38 a	483 a	3.04 a	4.46 c	36 a	155 a	160 a	1394 a	51 a	35 a
Broad bean blooming** on April 1, 2019										
Control	7.28 a	688 a	0.54 a	51.3 a	39 a	317 a	182 a	2028 a	228 a	93 a
Swede	7.36 a	728 a	1.10 a	31.4 a	44 a	292 a	183 a	2328 a	221 a	66 a
Turnip	7.34 a	649 a	0.55 a	39.9 a	42 a	235 a	179 a	1970 a	275 a	110 a
Chilli pepper harvest on October 22, 2019										
Control	7.74 a	226 a	4.06 a	8.5 a	33 a	92 a	145 a	2019 a	29 a	31 a
Swede	7.71 a	405 a	3.97 a	22.7 b	33 a	143 b	157 a	1986 a	86 b	43 a
Turnip	7.55 a	190 a	5.95 a	11.4 a	31 a	104 a	142 a	1775 a	42 ab	24 a

*for explanation see Table 3, ** K_2SO_4 fertilisation, EC – electrical conductivity

Fig. 1. STN70 tunnel during the flowering period of broad beans (April 14, 2019); the soil in plant rows was mulched with sorghum straw, nodules on the roots of broad beans grown after turnips for green manure in 2018

In the present experiment, it was observed that broad bean plants grown on plots with cover crops produced a significantly higher leaf biomass during the flowering period (Tab. 5). On the other hand, higher root weight was recorded for broad beans cultivated after the turnip cover crop compared to the control and swede cultivation. The most extended shoot length was determined for broad beans planted on the plot after turnip cultivation. The roots of broad beans grown after cover crops had a significantly higher number of root nodules than the control roots (Tab. 5). The highest number of these structures were observed in the configuration with turnip as a cover crop.

The foliar analysis of broad bean showed a significantly higher nitrogen content (4.11% DM) in leaves sampled from turnip treatment compared to the control and swede cultivation (Tab. 6). Additionally, the samples contained higher sulphur and copper contents compared to the control – moreover, cover cropping elevated boron levels in broad bean leaves compared to the control plants.

Soil microbiological analysis. A significantly higher number of bacteria was found in the soil collected during broad bean flowering on the plots 12 months after cover crop cultivation compared to the control plot (Tab. 7). The highest number of mesophilic bacteria was determined in the soil where turnip was grown as a cover crop (7.6 × 107 cfu g^{-1}). It should be noted that turnip biomass obtained in the tunnel and introduced into the soil was significantly higher than that of swede (Tab. 2). The abundance of bacteria in soils sampled from the control plots and plots sown with swede in 2018 was 2.0 105 cfu g^{-1} and 3.2 107 cfu g^{-1}, respectively. The soil where swede was grown was characterised by a considerably lower bacterial population (57%) compared to the plot where turnip was used as the cover plant. In addition, in the soil analysed after the cultivation of chilli peppers, another crop rotation in the tunnel in 2019, the highest count of bacteria was found (8.6 × 107 cfu g^{-1} DM soil) in the plot where turnips were grown in 2018. The lowest amount of bacteria was determined in the control variant – 4.0 × 107 cfu g^{-1} DM soil.

Soil analyses conducted during broad bean flowering showed that the highest number of fungi (2.0 × 104 cfu g^{-1} DM soil) was found on the plot with swede as the cover crop, both in relation to the control and turnip cultivation (Tab. 7). The count of fungi in the soil sown with turnip was determined at 8.2 × 103 cfu g^{-1}, while in the control soil covered with black foil during cover crop cultivation, the fungal count was determined at 3.7 × 103 cfu g^{-1}. Mean separation by two-way analysis, Duncan's test at p = 0.05. Means followed by the same letter are not significantly different.

Species from the family *Brassicaceae*, including turnips and swedes, contain glucosinolates. When

Table 5. Selected biological parameters for broad bean (*Vicia faba* L.) plants in an organically managed tunnel (June 18, 2019)

Treatment	Leaf biomass (g)	Root biomass (g)	Root length (cm)	Shoot length (cm)	Nodules number
Control	60 a*	16 a	25 a	58 a	138 a
Swede	84 b	16 a	27 a	63 b	245 b
Turnip	89 b	25 b	28 a	60 a	351 c

Table 6. Nutrient elemental content in the leaves of organically managed broad bean (*Vicia faba* L.) grown in high tunnel (2019; n = 2)

Treatment	N	Ca	K	Mg	P	S
Control	3.62 a	2.94 a	3.40 a	0.53 a	0.14 a	0.16 a
Swede	3.70 a	3.27 a	3.66 a	0.56 a	0.14 a	0.18 ab
Turnip	4.11 b	2.68 a	3.68 a	0.53 a	0.16 a	0.21 b
	B	Cu	Fe	Mn	Mo	Zn
Control	5.5 a	10.4 a	133 a	97 a	0.38 a	168 a
Swede	8.5 b	10.7 a	131 a	93 a	0.75 a	146 a
Turnip	6.4 b	16.9 b	133 a	93 a	1.12 a	181 a

See Table 3 for an explanation

Table 7. Total number of bacteria and fungi in the soil after cover crop treatment (2019; n = 2)

Treatment	Crop plant	Total number	
		Bacteria (cfu g^{-1} DM soil)	Fungi (cfu g^{-1} DM soil)
Control	broad bean April 1, 2019	$2.0 \cdot 10^5$ a	$3.7 \cdot 10^3$ a
Turnip		$7.6 \cdot 10^7$ c	$8.2 \cdot 10^3$ b
Swede		$3.2 \cdot 10^7$ b	$2.0 \cdot 10^4$ c
Control	chilli pepper harvest October 22, 2019	$7.4 \cdot 10^6$ a	$3.2 \cdot 10^3$ a
Turnip		$8.6 \cdot 10^7$ c	$4.8 \cdot 10^3$ b
Swede		$4.0 \cdot 10^7$ b	$4.6 \cdot 10^3$ b

Table 8. Most probable number (MPN) of ammonifiers, nitrifiers and denitrifiers after cover crop treatment (2019; n = 2)

Treatment	Crop plant	MPN (g^{-1} DM soil)		
		Ammonifiers	Nitrifiers	Denitrifiers
Control	broad bean April 1, 2019	$4.3 \cdot 10^6$ a	$2.3 \cdot 10^5$ a	$2.8 \cdot 10^4$ a
Turnip		$5.6 \cdot 10^6$ ab	$3.6 \cdot 10^5$ ab	$3.8 \cdot 10^4$ ab
Swede		$6.0 \cdot 10^6$ b	$3.9 \cdot 10^5$ b	$6.4 \cdot 10^4$ b
Control	chilli pepper harvest October 22, 2019	$7.5 \cdot 10^6$ c	$3.1 \cdot 10^5$a	$2.8 \cdot 10^4$ a
Turnip		$2.3 \cdot 10^6$ a	$6.2 \cdot 10^5$ c	$8.0 \cdot 10^4$ ab
Swede		$5.0 \cdot 10^6$ b	$5.7 \cdot 10^5$ b	$8.8 \cdot 10^4$ b

Mean separation by two-way analysis, Duncan's test at p = 0.05. Means followed by the same letter are not significantly different hydrolysed, glucosinolates produce compounds with toxic properties to plants, fungi, nematodes, and certain insects. Specifically, the hydrolysis of 2-propenyl glucosinolate produces volatile isothiocyanate compounds, which possess anti-fungal properties [Haramoto et al. 2004, Gieske et al. 2016]. The introduction of Brassica plants into the soil inhibited the growth of various soil-borne pathogens affecting potatoes, including *Rhizoctonia solani*, *Phytophthora erythroseptica*, *Pythium ultimum*, *Sclerotinia sclerotiorum* and *Fusarium sambucinum*, which consequently reduced the incidence of potato seedling diseases by 40–83% [Brennan and Acosta-Martinez 2017]. However, in the present experiment carried out in a high tunnel environment, the introduction of turnip and swede biomass into the soil did not result in a significant reduction in the number of fungi (Tab. 7). After the cultivation of chilli peppers in the tunnel in 2019, there was a decrease in the abundance of fungi in all the examined plots compared to the number determined during the flowering of broad beans.

The effect of cover plants on the most probable number (MPN) of ammonifiers, denitrifiers and nitrifiers in the soil is presented in Table 8. The MPN of ammonifiers in all tunnel facilities was estimated at 106 cfu g^{-1} DM soil.

An upward trend was observed in the number of ammonifiers in the control plot after chilli pepper cultivation, with a 70% increase in relation to the number determined during broad bean flowering. Conversely, on the plots where cover crops were grown, after the chilli pepper harvest, the abundance of ammonifying bacteria decreased by 58% on the turnip plot and by 15% on the swede plot. The lowest number of these bacteria was found on the plot previously sown with turnips (2.3 × 106 cfu g^{-1} DM soil). At the same time, an increase in the number of nitrifiers and denitrifiers was observed in the soils from these plots.

During the period of broad bean flowering, the number of nitrifiers in the soil was determined to be 105 cfu g^{-1} DM soil (Tab. 8). The control plot contained the smallest population of these bacteria, i.e., 2.3 × 105 cfu g^{-1} DM soil, which was significantly lower compared to the swede treatment (3.9 × 105 cfu g^{-1} DM of soil). It is important to note that high concentrations of NO_3-N were also detected in the soils of all plots during this period.

After the cultivation of chilli peppers, the soil where turnip was used as a cover crop exhibited the highest number of nitrifiers compared to the control. At the same time, the soil with turnip as a cover crop had significantly higher levels of NO_3-N than in other experimental configurations.

An increase in the number of nitrifiers was observed after chilli pepper cultivation on all experimental plots in relation to the analyses performed during broad bean flowering. On the plot where turnip was grown as a cover crop, there was a 40% increase in the number of nitrifiers compared to the previous cultivation. It should be noted that the amount of substrate (NH4-N) for these microorganisms in this particular period was high compared to the other time points of soil chemical analysis.

The number of denitrifying bacteria was determined at the level of 104 cfu g^{-1} DM soil. The control plot had the lowest number of these bacteria (2.8 104 cfu g^{-1} DM soil) during broad bean flowering and after chilli pepper cultivation, followed by the plot sown with turnips and swede as cover plants (Tab. 8). On both analysed dates, statistically significant differences were recorded only for the number of denitrifiers on the plot after the turnip cultivation compared to the control plot. An increase in the number of denitrifiers was observed in the 2019 crop rotation cycle in the high tunnel following the cultivation of chilli peppers. Specifically, there was a 30% increase in the swede plot and a 50% increase in the turnip plot.

DISCUSSION

The use of non-leguminous broadleaf plants, such as swede and turnip, as cover crops in a protected high tunnel environment between October and April resulted in the production of high biomass and the supply of nitrogen ranging from 75 to 110 kg ha^{-1}. Studies by Thorup-Kristensen et al. [2003] and Robacer et al. [2016] have shown that nitrogen uptake by cover crops can vary significantly, with non-legume cover crops typically accumulating around 10 kg N ha^{-1} to legume cover crops accumulating up to 200 kg N ha^{-1}. This high variability is mainly determined by factors such as changing climatic conditions, root growth, contact with available soil nitrogen, and the amount of nitrogen available in the soil. Based on field experiments, Grieske et al. [2016] reported that nitrogen accumulation in the biomass of five brassica cover crops planted in autumn was generally higher than 70 kg ha^{-1} (ranging from 31 to 136 kg ha^{-1}). Similar results can be found in a study by Dean and Weil [2009].

The present findings demonstrated that the residue input from cover crops had a significant impact on soil organic carbon content, the prevention of plant available nitrogen, and the increase of wet aggregate stability. A review by Haruna et al. [2020] and Blanco-Canqui and Ruis [2020] indicated that CCs could lower soil bulk density by approximately 4% compared to untreated soil. One of the critical benefits of cover cropping is the build-up of soil organic matter (SOM). The decomposition of CC residues contributes to the formation of SOM, which can have physical and chemical preservation effects on silt and clay fractions, enhancing their stability and resistance to decay. Organic matter and microbial biomass in fine-textured soil may, therefore, show a greater and more rapid response to cover crop approaches [Cherr et al. 2006]. The current results are consistent with data from 139 plots at 37 different sites compiled by Poeplau and Don [2015], who showed that, overall, cover crop treatments generated significantly higher SOC stocks compared to reference croplands. Most of the C entering the SOM comes from below-ground sources, including root biomass and rhizodeposits, while C from plant shoots produced via respiration is mostly missing in the soil. Organic carbon from lignified roots (polymers, such as lignin derivatives and long-chain lipids) of non-legume cover crops may persist longer in the soil, increasing the long-term root contributions to SOM and possibly also soil organic nitrogen [Cherr et al. 2006, Brenna and Acosta-Martinez 2017, Koudahe et al. 2022]. Berg [2014] found that high N concentrations in plant residues can only affect cellulose decomposition in the early stages. The same high nitrogen content in plant biomass can retard the mineralisation of lignin in cell walls at a later stage, and this effect increases with growing N concentrations. Austin et al. [2017] indicated the significance of CC roots compared to shoots and highlighted the role of cover crop rhizodeposition as an essential source of soil C, accounting for approximately 33% of the total below-ground cover crop C inputs.

The high tunnel experiment conducted in this study demonstrated that the use of cover crops (CCs) resulted in the highest water-stable aggregate index in the soil. The microbial decomposition of plant residues releases energy and nutrients that allow the functioning of soil organisms, including nutrient cycling and soil aggregation [Saleem et al. 2020]. Water-stable aggregates often influence soil physical properties, such as soil erodibility and health. The presence of cover crops, particularly those from the family Brassicaceae, has been reported to improve soil aggregation and water penetration in fine-grained soils [Haruna et al. 2020, Blanco-Canqui and Ruis 2020]. In some agroecosystems, cover crops have been observed to improve soil aggregation by increasing soil organic carbon content [Saleem et al. 2020], which was also confirmed in the present study. Soils rich in organic carbon or organic matter have a stable structure. The microbial activity in the soil, including the activities of fungi and bacteria, can also influence soil aggregation. Fungal hyphae and bacterial secretions, such as polysaccharide components, can contribute to better soil aggregation by promoting the binding of soil particles [Koudahe et al. 2022].

Organic residues inside soil aggregates are physically protected in areas with low microbial activity [Kallenbach et al. 2016]. The results of Han et al. [2021] indicated the importance of soil aggregates in nitrogen cycle transformations, microbial metabolism and taxonomic composition. Soil structure can be considered an indicator of the "soil ecological status" [Saleem et al. 2020], acting as an integrating property that reflects the vital soil functions: providing habitat for diverse biological activity, facilitating the production of biomass, and responding to extreme perturbations [Schlüter et al. 2011].

Research by Koudahe et al. [2022[and Scavo et al. [2021] have demonstrated that cover crops have the potential to improve not only soil organic carbon and nitrogen accumulation but also enhance the availability of essential nutrients such as P, K, Ca, Fe and Mg in specific soil types and climatic conditions. The total nitrogen content in mineral soils typically ranges from 0.02% to 0.3%, corresponding to approximately 600–9000 kg N ha^{-1} in the arable layer; however, only a small fraction, around 2%, exists in inorganic forms [Barker and Pilbeam 2017, Grzyb et al. 2021]. Despite its low proportion, the inorganic fraction serves as the primary source of nitrogen for plant nutrition.

Throughout the year, fluctuations in the concentrations of both ammonium (NH_4^+) and nitrate (NO_3^-) ions are observed in the soil, with more remarkable changes in nitrate content compared to ammonium [Robertson and Vitousek 2009]. Some of the ammonium ions can be firmly bound by clay minerals, illite, vermiculite and monmorillonite [Mooshammer et al. 2014], especially in fine-grained soils. The findings of Perkus et al. [2022] align with the present research, suggesting that cover crops can reduce the soluble nitrogen content in the soil compared to bare soil, indicating their role in nitrogen retention in the ecosystem.

A higher number of nitrogen cycle bacteria were found in the soils after CC cultivation compared to the control soils. It was particularly true for bacteria reducing NH_4^+-N and NO_3^--N. Microbial oxidation of NH_4^+ to NO_3^- through nitrite (NO_2^-), known as nitrification, can be carried out by ammonia-oxidising bacteria and ammonia-oxidising archaea [Farooq et al. 2022]. Recent studies have revealed that direct uptake of organic materials can significantly contribute to the available N nutrition for soil microorganisms [Scavo et al. 2021, 2022]. Microbial growth in soils can be limited by either energy (C) or nutrient (N) availability, or both, whereas declining C : N ratios from litter to soil indicate reduced C availability compared to N, and thus a decreasing N limitation. Microbial carbon use efficiency, i.e. the amount of C utilised for microbial growth relative to total C uptake, can have a direct impact on microbial residue production, but it can differ across resource gradients and microbial communities [Geisseler et al. 2010, Kallenbach et al. 2016, Brennan and Acosta-Martinez 2017, Grzyb et al. 2020, Koudahe et al. 2022].

Crop residues are valuable natural resources, and the return of organic plant material to the soil is a nutrient-conserving practice that promotes crop production and soil fertility. The results reported by Wang et al. [2018] showed that the incorporation of crop residues enhanced crop yield, increased N pool in the soil (by 10.7%), promoted microbial presence (by 34.9%), and improved crop growth (by 20.8%). The high tunnel experiment showed an active symbiosis of broad bean roots with *Rhizobium* bacteria [Jiang et al. 2021]. Root nodules were variable, with a slight red colouring, which could indicate the production of leghemoglobin, a protein that protects the nitrogenase complex from excessive oxygen concentrations [Jiang et al. 2021]. The rapid decomposition of legume roots, as explained by Franche et al. [2009], contributes to increased organic matter and nutrient contents in the soil and affects its aeration. Dinesh et al. [2006] reported that long-term cultivation of legume cover crops significantly improved N and C cycling facilitated by soil microorganisms, leading to higher levels of total organic C and N in the soil.

Romdhane et al. [2019] reported that modifications in soil properties due to cover crop management were associated with changes in the number of ammonia oxidisers and denitrifiers, but the overall bacterial abundance was not affected. In a 6-year field study conducted in high-input, tillage-intensive organic vegetable production systems, Brennan and Acosta-Martinez [2017] did not find a correlation between cover crop shoot biomass and microbial biomass or SOC; however, they observed relatively significant differences in cumulative cover crop shoot biomass between mustard (30.5 t ha^{-1}), legume-legume rye (45.1 t ha^{-1}), and rye (42.9 t ha^{-1}). These results provide evidence that carbon (C) inputs from frequent cover cropping are the primary driver of changes in the soil food web. The ratio of fungal to bacterial indicators decreased over time, whereas indicators of invertebrates and Gram-positive bacteria increased. We observed that soils with the highest SOC concentrations exhibited the greatest bacterial and fungal abundance. In most environments, fungi represent the majority of the primary population that decomposes plant materials. Fungi secrete enzymes (e.g., cellulose) to digest complex organic compounds into bioavailable forms. Fungal genera known for their proteolytic abilities include *Aspergillus*, *Mucor*, *Cladosporium*, *Botrytis* and *Trichoderma* [Grzyb et al. 2020]. Alterations in fungal abundance and community structure mainly caused changes in soil organic carbon (SOC) and nutrient contents. Similarly, plants allocate photosynthetic products into the rhizosphere to provide nutrition for microorganisms [Xiang et al. 2020]. The latter authors reported that 29 years of mineral N fertilisation decreased soil fungal abundance by 44%, while long-term manure application increased fungal abundance by 79%. Decomposition of organic materials with a higher C : N ratio is favoured by fungi, while bacteria decompose more readily materials with a low C : N ratio [Brennan and Acosta-Martinez 2017]. Therefore, the higher C : N ratio of sorghum straw used as organic mulch in November 2018 likely made this material more accessible to fungal rather than bacterial decomposition. It could explain the decrease in indicators for saprophytic fungi observed in 2019 after the chilli pepper harvest.

Most soil bacteria function as decomposers, breaking down simple carbon compounds, such as fresh plant litter and root exudates. N-cycling includes microbiological processes such as nitrogen fixation, mineralisation (decay), nitrification and denitrification [Hayatsu et al. 2008]. The final step in the mineralisation of simple organic nitrogenous substances with the release of NH_4^+-N is known as ammonification, and numerous bacteria (*Bacillus* sp., *Clostridium* sp., *Proteus* sp., *Pseudomonas* sp. and others), as well as actinobacteria, participate in this process [Grzyb et al. 2020].

During the flowering period of broad bean (12 months after CC cultivation), the present study revealed a significantly higher number of ammonifiers in the plot after swede cultivation compared to the control plot. Although not statistically significant, the concentration of NH_4^+-N in the soil of the swede plot was twice as high as that of the control plot during this period (Tab. 8). On the other hand, microbiological analyses conducted during the second period (after pepper cultivation, 19 months after CCs) demonstrated a significantly higher abundance of ammonifying bacteria on the control plot (7.5×10^6 cfu g^{-1} DM of soil) in comparison to the remaining objects. At the same time, the control soil analysed in this period showed a low content of mineral nitrogen. In soils sown with CCs, this could indicate the end of the biomass decomposition process, including underground brassica residues, and suggested that the effect of plant roots is often transient. Soil bacteria can grow and adapt rapidly to changes in soil properties [Schloter et al. 2018]. In the processes of ammonification, catabolism results in the release of energy for anabolic activity. These processes require the uptake and utilisation of mineral N (immobilisation) by decomposer organisms [Geisseler et al. 2010]. However, a significant amount of this N is eventually released back into the soil through the lysis of microbial tissues. Therefore, the degradation of microbial tissues is of great importance in terms of the final release of N originally bound in plant residues to the soil [Farooq et al. 2022]. Determination of the mineral nitrogen content in the soil conducted 19 months after CC cultivation revealed the highest concentration of Nmin on the plot after swede cultivation (26.7 mg NH_4-N + NO_3-N dm^{-3}) compared to turnip (17.4 mg) and control (12.6 mg) plots (Tab. 4). An increased concentration of NH_4-N in the soil was observed on the plots sown with cover crops during this period compared to the amount of this form determined after broad bean harvest (Tab. 8). It should be noted that carbon-rich sorghum straw was also decomposing in the soil during the flowering period of broad bean, as were organic compounds from the decomposition of lignified CC roots and rhizodeposits left in the soil by growing crops. When the C : N ratio is high, as in the case of sorghum straw, decomposers can retain soil N and release excess C through CO_2. As decomposition progresses and the C : N ratio decreases, microbial growth becomes restricted by C limitation, and excess N is released through N mineralisation [Grzyb et al. 2020].

In managed soils, NH_4^+ is typically rapidly oxidised to NO_3^- in a reaction known as nitrification (biological oxidation of NH_4^+-N) [Geisseler et al. 2010]. Numerous genera of autotrophic bacteria, including *Nitrospira*, *Nitrosomonas* or *Nitrosolobus*, are capable of oxidising ammonium to nitrate, although *Nitrobacter* appears to be the dominant or the only nitrate oxidiser [Grzyb et al. 2020]. Taylor and Bottomley [2006] reported that the addition of NH_4^+-N to the soil solution increased NH_4^+ levels, which stimulated NO_2^-, particularly by species like *Nitrosomonas europaea* (3.3-6.6-fold increase) and *Nitrosospira* sp. AV (1-2.1-fold increase). Under certain conditions, NO_3^- ions may undergo denitrification, a process facilitated by various soil microorganisms not as specialised as nitrifiers [Grzyb et al. 2020]. The majority of denitrifying bacteria are heterotrophic aerobes that can switch rapidly to denitrifying metabolism when the oxygen levels decrease [Farooq et al. 2022]. There are also aerobic denitrifiers that are capable of denitrification in the presence of oxygen. Among the frequently encountered species known for their metabolic adaptability are *Alcaligenes* and *Pseudomonas* species. Heterotrophic denitrifiers require organic carbon for cell growth and as an electron donor for denitrification [Rajta et al. 2020].

The application of turnip and swede cover plants intensified the biological activity of the soil and enhanced microbiological processes, such as denitrification. Plant root secretions, which contain easily degradable carbon compounds, have been found to stimulate the activity of denitrifying microorganisms [Grzyb et al. 2020]. Brennan and Acosta-Martinez [2017] and Patkowska et al. [2016] reported that the type and frequency of cover cropping type altered the relative abundance of some bacteria, such as *Pseudomonas*, which are capable of simultaneous heterotrophic nitrification and aerobic denitrification. However, Brennan and Acosta-Martinez [2017] noted a decline in the abundance of *Pseudomonas* in plots with brassica cover crops (mustard) while an increase in plots with legume-rye and rye alone.

We observed that soils with the highest SOC concentrations were associated with the greatest abundance of bacteria and fungi. Soil microbes produce chemically diverse, stable SOM. Recent work suggests that the most stable soil C originates from microbial conversion or represents dead microbial biomass. Kallenbach et al. [2016] have argued that SOM accumulation is primarily determined by different microbial communities rather than soil mineralogy. Specifically, soils with higher fungal abundance and more efficient microbial biomass production tend to exhibit greater accumulation of microbial-derived organic matter. Changes in plant residues can affect microbial physiology, thereby affecting the input of microbial residues into the soil. The distinct microbial communities observed in soils treated with cover crops likely reflect repeated exposure to different substrates, which favoured the selection of different microbial communities with varying carbon use efficiency. The results of a study by Brennan and Acosta-Martinez [2017] have provided evidence for the significant role of carbon inputs from regular cover cropping in driving changes in the soil food web and enhancing soil health in organic vegetable production systems

CONCLUSION

Our investigation focused on the potential benefits of brassica cover crops for soil health in cold tunnel cropping systems within temperate climates. Specifically, we examined the ability of cover crops to establish and produce substantial biomass during winter in high tunnels managed organically. We found that non-leguminous broadleaf swede (*Brassica napus* subsp. *napobrassica* (L.) Jafri) and turnip (*Brassica rapa* subsp. *Rapifera* (Metzg.)), used as cover crop species between October and April in the slightly warmer and protected high tunnel environment, produced high biomass and provided between 75 and 110 kg ha^{-1} N. Furthermore, these cover crops contributed to increased soil carbon and nitrogen levels, as well as improved availability of sulphates. In summary, our study demonstrated the substantial impact of cover crop residues on various soil properties. These included the accumulation of soil organic carbon, retention of plant-available nitrogen, and improvement of soil physical properties, particularly wet aggregate stability. Notably, we observed a positive correlation between higher soil organic carbon concentrations and increased abundance of bacteria and fungi.

Organic plant residue can alter microbial physiology and consequently impact microbial residue content in the soil. The use of turnip and swede cover plants intensified the biological activity of the soil and increased the activity of various microbiological processes, including ammonification, nitrification and denitrification. We observed that the stimulating effect on bacterial counts persisted longer as time passed since the cultivation of brassica cover plants, with the most potent effect observed for the soil where the turnip was grown.

Our research highlights the significance of cover cropping practices in high tunnels as crucial drivers of both the composition of the entire bacterial community and the abundance of N-cycling microbial associations.

Future research should focus on investigating and quantifying specific changes in the soil microbiome that occur in response to the implementation of cover crops in sheltered farming systems. Utilising microbial network analyses is a promising strategy to identify and understand the potential role of specific microbial taxa in vegetable growing systems

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