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ORIGINAL ARTICLE

Analysis of the number of hemimetabolous insects at variable wheat sowing densities – the role of nitrogen in the plant and soil

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Abstract

This work investigated the influence of nitrogen compounds on soil–plant–insect interactions. The study aimed to assess the effect that cultivation of two spring wheat cultivars (*Triticum sphaerococcum* Percival and *Triticum persicum* Vavilov) at various plant densities (400, 500 and 600 grains · m^{-2}) under organic and conventional farming had on the abundance of mimetabolous insects in the context of selected chemical and biochemical soil properties. The soil was assayed for its content of organic carbon (SOC), total nitrogen (STN), nitrate (SNO_3^-) and activity of proteases (PRO) – an enzyme involved in N transformation. The aboveground part of spring wheat was assayed for the content of total nitrogen (TNC) and nitrate nitrogen (TNO_3^-). Spring wheat variety and plant density both determined the SOC and STN content. The tested soil was characterized by low SOC content. PRO activity was positively correlated with SOC and STN contents, which indicated the role of this enzyme in organic matter cycling. Thysanoptera, Aphididae and Miridae were found to prefer spring wheat grown under organic farming. However, the numbers of these pests did not pose a threat to the growth and development of the host plant. Hemimetabolous insects were far less abundant on *T. persicum* than on *T. sphaerococcum*.

Keywords: insects, nitrogen nitrate, organic and conventional farming, organic carbon, protease, wheat

Introduction

Wheat is the main cereal grown in Poland. To maximize wheat yields, intensive nitrogen fertilization and plant protection with pesticides are usually used (Tudor *et al.* 2023). However, the intensification of production is having negative environmental effects, including soil degradation (Pikuła 2015; Jaskulska *et al.* 2020) and an increased abundance of phytophagous insects (Lemanowicz *et al.* 2023b). Therefore, in wheat production, special attention is being paid to optimally use the soil's natural production capacities. The provision of nutrients and any adjustment of plant protection against pathogens and pests also need to consider the requirements of each varietal. This allows for effective management of both nutrients and pest protection (Lemanowicz *et al.* 2023b). The basic indicator of soil quality determining physicochemical soil properties is organic matter content (OM) (Debska *et al.* 2020; Hayatu *et al.* 2023). Intensive human activity is the dominant factor in soil degradation through its effect on the decomposition of TOC and TN (Pikuła 2015). This causes a general decrease in the content of these macro-elements in the soil (Illiger *et al.* 2019). Wheat crop productivity depends largely on N bioavailability in the soil. Therefore, N demand is the most important feature of crop production (Anas *et al.* 2020).

Research by Yan *et al.* (2016) has shown that, over the course of a year, soil processes provide plants with more N than fertilization. Therefore, the soil's ability to retain N plays a fundamental role in supplying this element to plants. Competition for nitrogen increases with plant density, reducing the amount available to individual plants (Tollenaar *et al.* 2006). In cereal crops, available N occurs mainly in the form of nitrate (NO_3^-) and ammonium (NH_4^+). The ammonium form accounts for an average of 10% of the NO_3^- concentration in soil, making it the dominant form of N available to plants. The sources of NO_3^- in the soil decompose plant residues, organic, natural, or mineral fertilizers, and root exudates. Plants can obtain more nitrogen NO_3^- than is needed for assimilation and storage, e.g., in leaf vacuoles. They are then available for use when N levels are low (Anas *et al.* 2020). Nitrogen also occurs in organic form, mainly as amino acids (Giordano *et al.* 2021). Natural and anthropogenic processes occur in the soil that reduce the availability of nitrogen. N losses can limit many functions related to carbon (C), nitrogen (N) and phosphorus (P) cycling in soil (Debska *et al.* 2020; Hayatu *et al.* 2023). In nitrogen biogeochemistry, proteases play an important role, hydrolyzing peptide bonds (CO-NH) into polypeptides and amino acids (Naga Raju *et al.* 2017; Greenfield *et al.* 2021). The release of amino acids is the first phase of N mineralization and an essential step in nitrogen uptake by plants (Sardans *et al.* 2008). Proteases are released into the soil by the action of microorganisms or by plant roots (Greenfield *et al.* 2020). The activities of enzymes, including proteases, are used as soil quality indicators to assess soil functioning (Lemanowicz *et al.* 2023a). Protease activity has also been recognized as one of the rate-limiting steps in soil organic nitrogen (SON) mineralization (Fuji *et al.* 2020). Changes in soil enzyme activity usually indicate changes in the properties of edaphic factors, which can then affect plant growth (Yang *et al.* 2017).

Cereals, in particular wheat, are exposed to the attack of many pests during their growing season. The most important herbivores of monocotyledonous plants include hemimetabolous insects such as aphids (the cereal aphid – Sitobion avenae F. and the bird cherry-oat aphid – Rhopalosiphum padi L.) or the Miridae (Hemiptera), as well as insects belonging to the order Thysanoptera (Walczak 2007; Jandricic *et al.* 2014; van Emden and Harrington 2017). Lamparski (2020) reports that the bird cherry-oat aphid most frequently inhabited wheat during its earing, thrips and Miridae during its flowering, and the cereal aphid during milk-dough maturity.

Insect abundance depends on many factors, both biotic and abiotic. The most important factor regulating interactions between herbivorous insects and their hosts is the chemical composition of the host plants, which contain over 200,000 metabolites (Fernie 2007). In the interactions between phytophages and their hosts, nitrogen compounds (which are mainly of anthropic origin) are of particular importance (Throop and Lerdau 2004). Sowing density affects the moisture content of the field and the availability of nutrients, which affects the nutritional status of plants. Systematic inspection of a cereal crop makes it possible to rationally protect plants against pests, as control measures should only be taken when pest numbers exceed the adopted harmfulness threshold. This restraint makes grain production technology more environmentally friendly. It is believed that an increase in nitrogen content in plant tissues is beneficial for herbivores – it leads to an "improvement" in the chemical composition of the host plant. Nitrogen therefore has a beneficial effect on the development of insect populations (Bala *et al.* 2018).

Nitrogen compounds are important elements influencing plant growth but also affect insect communities. Rahman (2022) reports that plants receive N through their roots in the form of ammonia or nitrate. Nutritional quality and defense of plants that have a direct impact on herbivorous insects are altered by N fertilization and herbivorous insects can differentiate between plants that receive different applications of N fertilizer. Moreover, nitrogen fertilization affects many aspects of insects such as population dynamics, larval count, larval weight, feeding choice, and oviposition preference. Furthermore, predatory insect abundance, parasitization performance, and development of parasitoids on host insects are negatively affected by N fertilization. Wahsh *et al*. (2023) evaluated the influence of nitrogen fertilization levels on the population abundance of the main piercing-sucking insect pests that attack cucumber crops. The cotton aphid *Aphis gossypii* followed by the cotton whitefly *Bemisia tabaci* showed the largest average numbers at each nitrogen level. Meanwhile, the green sting bug *Nezara viridula*, the cotton mealybug *Phenacoccus solenopsis*, the onion thrip *Thrips tabaci* and the leafhopper *Empoasca decipiens* had the lowest average numbers in comparison to the other examined insect pests. Shan *et al.* (2021) reports that high nitrogen input raises the potential risk of rice pest outbreak. Wang *et al.* (2020) found that aphid abundance was reduced in the P0N1 treatment (lower nitrogen dose used in the experiment) on wheat.

To date, most research on plant–insect interactions in agricultural systems has focused on herbivores. Information on the indirect effects of soil nitrogen on insects is scarce. Any changes in plant chemistry are likely to have significant direct and indirect effects on insect populations. Therefore, comprehensive studies were undertaken on nitrogen transformations in the soil, its content in the aboveground parts of spring wheat, and its influence on plant–insect interactions.

The study aimed to determine the effect that cultivation of two spring wheat varieties (*Triticum sphaerococcum* Percival and *Triticum persicum* Vavilov) under organic and conventional systems had on the abundance of hemimetabolous insects in terms of selected chemical and biochemical properties of the soil.

Materials and Methods

Experiment design

The research was conducted in 2018–2020 on the premises of the RZD (Agricultural Experiment Station) in Mochełek, Kuyavia-Pomerania Voivodeship, Poland (53°13'N, 17°51'E, 95 m a.s.l) and in the fields of a private organic farm in Grabina Wielka, Wielkopolska Voivodeship, Poland (52°11'N; 18°80'E, 102 m a.s.l.). Spring wheat plants (*T. sphaerococcum* and *T. persicum*) were grown under two farming systems: organic (OF) and conventional (CF). Plant samples for testing were collected at the beginning of the flowering of wheat. For a description of plots and the conducting of field experiments and soil properties (Mochełek, Grabina Wielka), see Lemanowicz *et al.* (2020) and Szczepanek *et al.* (2020). Also, for the characteristics of wheat varieties, sowing dates and weather conditions in Grabina Wielka, see Lemanowicz *et al.* (2020) and Szczepanek *et al.* (2020). The soil and weather conditions in Mochełek are presented in Szczepanek *et al.* (2022). In the organic farming system, two weeks before sowing, organic fertilizer Bioilsa was applied (N 12 kg, P_2O_5 10 kg, K_2O 26 kg; MgO 4 kg, SO_3 20 kg · ha^{-1}. In the conventional farming system, at the beginning of April, pre-sowing fertilization was performed: N 30 kg, P_2O_5 30 kg, and K_2O 50 kg · ha^{-1}. Additionally, N was applied at the beginning of stem shooting (mid-May), at a dose of 30 kg · ha^{-1}.

Soil analysis

The soil from the 0–20-cm layer was sampled between spring wheat rows using an Egner stick. Selected physicochemical properties of the soil were determined in air-dried soil samples sifted through a ø 2-mm sieve, namely:

- granulometric composition using a Mastersizer MS 2000 particle analyzer (Malvern Panalytical, UK);
- pH in 1M KCl measured potentiometrically (PN-ISO 10390, 1997);
- and content of soil organic carbon (SOC) and soil total nitrogen (STN) using a Vario Max CN analyzer from Elementar (Germany);
- the content of NO_3^- (SNO_3^-) in soil. Two grams of soil sample were mixed with 50 mL of a 1% solution of $KAl(SO_4)_2$ (Merck KGaA, Darmstadt, Germany) and thoroughly extracted. The extraction was conducted for 1 h. Ten mL of a 60% solution of $Al_2(SO_4)_3$ (Acros Organics, Waltham, MA, USA) was added to the filtrate and mixed directly before determination. Nitrate content was determined using KNO_3 standard curves (Merck KGaA, Darmstadt, Germany). A CX-721 multifunctional computerized apparatus (Elmetron, Zabrze, Poland) was used to determine nitrate content by the ion-selective potentiometric method (according to the CX-721 manufacturer's instructions for use and Baker and Thompson 1992). The measurement principle is based on the linear dependence of the electrode potential on the logarithm of ion activity in solution. The device was provided with a nitrate electrode, a double-junction reference electrode (the outer chamber was filled with 0.02 M solution of $(NH_4)_2SO_4$ (Merck KGaA, Darmstadt, Germany), a specific ion meter, and a pH/milovolt (mV) meter with a reading accuracy of 0.1 mV. The limit of determination was set at 30 mg · kg^{-1}, and the measurement error was about 15%, according to the matrix of the sample being measured.
- protease activity (PRO) was determined in fresh-sieved (<2 mm) soils by the method of Ladd and Butler (1972). According to this method, tyrosine (Tyr) concentration was determined in soil samples after incubation with sodium caseinate. Absorbance was measured by spectrophotometer at λ = 680 nm.

Plant analysis

The wheat plants were cut and frozen. The frozen wheat samples were placed for 48 h in a Whirlpool AFG 6402 E-B (Pero, Milano, Italy) freezer at −18°C. Next, the freeze-drying process was conducted in a CHRIST ALPHA 1–4 LSC (Osterode am Harz, Germany). Freeze-dryer operating parameters were as follows: condenser temperature 55°C, vacuum 4 kPA at 20°C. The wheat samples were dried to a constant weight when the final water content of the material was less than 2%. Drying was conducted for 24 h. The dried samples were ground (particle size 0.3–0.5 mm) using an Ultra-Centrifuge Retsch ZM 100 laboratory grinder (Retsch GmbH, Haan, Germany). The milled samples were placed in sealed bags LDPE and stored in the dark until (vacuum desiccator) laboratory testing.

In the materials thus prepared, the content of total nitrogen in wheat (WTN) (Sáez-Plaza and Navas 2013) was determined based on a modified Berthelot reaction (Skalar method). In brief, after dialysis against a buffer solution of pH 5.2, ammonia in the sample is chlorinated to monochloramine, which reacts with salicylate to form 5-aminosalicylate. Following oxidation and oxidative coupling, a green complex is formed. The absorption of the complex is measured at 660 nm (Skalar SANplus flow analyzer, Breda, The Netherlands).

Two grams of freeze-dried wheat (Baker and Thompson 1992) were mixed with 50 mL of 1% $KAl(SO_4)_2$ and the procedure for determining nitrates in the soil as given above was followed. Nitrate contents were expressed as dry matter ($mg \cdot kg^{-1}$ d. m.).

Insect experiments

Insects were collected at the flag leaf stage of spring wheat (BBCH 39). Twelve sweeps of an entomological net (ø 30 cm) were made on each of the experimental plots (1 strike = 2 m^2 of experimental plots). The sums of particular groups (i.e., Thysanoptera, Aphididae and Miridae) caught with the entomological net were expressed as individuals per plot (ind. $24 \cdot m^{-2}$). The results are presented as insect density, i.e., the sum of particular groups caught with the use of the entomological net – in individuals per plot. Entomological material was determined according to the keys of Müller (1976), Korcz (1994) and Zawirska (1994).

Statistical analysis

The data obtained from the analyses were subjected to two-factor analysis of variance using Tukey's HSD *post-hoc* test at the significance level of $p = 0.05$. The normality of the distribution of the analyzed parameters was tested using the Shapiro–Wilk normality test. The first factor was spring wheat species (*Triticum sphaerococcum Perc.* and *Triticum persicum*) and the second was sowing density (400, 500 and 600 grains $\cdot$ m^{-2}). The results were expressed as an arithmetic mean with standard deviation (±SD). To determine the relationships between the studied parameters, correlation coefficients were calculated using the PAST 4.13 program (Hammer *et al.* 2001).

Results and Discussion

The granulometric compositions of the soils were highly homogeneous. They were classified into one granulometric group: sandy loam (USDA 2006). Under both systems, the granulometric composition was dominated by the sand fraction, which ranged from

Table 1. Selected physicochemical properties of soils

Wheat species	OF			CF		
	Sowing density [grains · m^{-2}]					
	400	500	600	400	500	600
	Sand [%]					
Triticum sphaerococcum	63.22 ± 0.47[1]	63.71 ± 0.35	63.46 ± 0.45	52.78 ± 0.16	52.78 ± 0.38	50.12 ± 1.35
T. persicum	66.44 ± 0.24	64.56 ± 0.41	65.99 ± 0.92	54.08 ± 0.31	54.41 ± 0.16	51.20 ± 1.52
	Silt (%)					
T. sphaerococcum	32.71 ± 0.43	31.96 ± 0.31	31.94 ± 0.52	41.96 ± 0.15	41.75 ± 0.38	44.19 ± 0.34
T. persicum	29.27 ± 0.22	31.11 ± 0.36	29.68 ± 0.89	40.82 ± 0.24	40.68 ± 0.16	43.11 ± 0.15
	Clay (%)					
T. sphaerococcum	4.39 ± 0.07	4.33 ± 0.05	4.59 ± 0.07	5.26 ± 0.01	5.47 ± 0.05	5.69 ± 0.03
T. persicum	4.29 ± 0.03	4.33 ± 0.05	4.32 ± 0.06	5.11 ± 0.07	4.91 ± 0.03	5.70 ± 0.02
	pH in 1 M KCl					
T. sphaerococcum	4.95 ± 0.21	5.63 ± 0.65	5.18 ± 0.82	7.54 ± 0.33	7.53 ± 0.65	7.53 ± 0.58
T. persicum	4.63 ± 1.15	4.95 ± 1.1	5.13 ± 0.91	7.52 ± 0.80	7.45 ± 1.55	7.46 ± 0.61

OF – organic farming; CF – conventional farming; [1]SD – standard deviation

63.12 to 66.44% for organic farming and from 50.12 to 54.41% in CF (Table 1). The pH in 1 M KCl in soil samples ranged from 4.63 to 5.63 (acidic and slightly acidic soil) under OF and from 7.45 to 7.54 (alkaline soil) under CF (Table 1).

The soil organic carbon (SOC) content under OF ranged from 6.86 g · kg^{-1} to 9.62 g · kg^{-1} (average 8.34 g · kg^{-1}) (Table 2). Under CF, the SOC content was about 20% higher (from 9.22 g · kg^{-1} to 11.58 g · kg^{-1}). The experimental factors significantly influenced the SOC content in soil. Under OF, the content was significantly higher in the soil in which *T. persicum* (8.78 g · kg^{-1}) was cultivated than in *T. sphaerococcum* (7.90 g · kg^{-1}). A similar relationship was found for CF. For the two farming systems, the highest significant OC content was obtained in the soil sown with a density of 400 grains · m^{-2} under OF and 500 grains · m^{-2} under OF. According to the European Soil Base (ESB) (Gonet 2007), the tested soil was characterized by very and low OC content something is missing. According to Debska *et al.* (2020), variations in OC content depend on soil type, crop rotation, and/or the amount of crop residues, among other factors. The soil total nitrogen (STN) content in the soil was significantly dependent on both wheat variety and sowing density (Table 2). The STN content was higher in soil under OC (average 1.09 g · kg^{-1}) than under CF (average 0.82 g · kg^{-1}). The STN content in soils depends on the quantity and quality of organic matter, as well as on its degree of decomposition, which is indicated by the carbon-to-nitrogen ratio. It is also one of the parameters characterizing soil fertility. The SOC/STN ratio was slightly wider in the soil under CF (8.31–10.59) (Fig 1A) compared to OF (8.81–11.48)

Table 2. Content of organic carbon, total nitrogen and nitrate nitrogen SNO_3^- in soil

Wheat species I factor	OF				CF			
	Sowing density [grains · m^{-2}] – II factor							
	400	500	600	mean	400	500	600	mean
	SO [g · kg^{-1}]							
Triticum sphaerococcum	*a* 9.24 a ± 0.006[1]	*b* 7.62 b ± 0.024	*c* 6.86 b ± 0.013	7.90 B ± 0.105	9.22 a ± 0.013	11.14 a ± 0.019	*b*9.91 b ± 0.026	10.09 B ± 0.085
T. persicum	*b* 9.13 a ± 0.011	*a* 9.62 a ± 0.010	*c* 7.59 a ± 0.008	8.78 A ± 0.090	*c* 9.24 a ± 0.017	10.80 b ± 0.011	*a*11.58 a ± 0.011	10.54 A ± 0.102
mean	9.19 *A* ± 0.10	8.62 *B* ± 0.108	7.23 *C* ± 0.041	8.34 ± 0.106	9.23 *C* ± 0.014	10.97 *A* ± 0.023	10.75 *B* ± 0.091	10.32 ± 0.095
$HSD_{0.05}$	I = 0.04; II = 0.16; II/I = 0.22; I/II = 0.15				I = 0.21; II = 0.17; II/I = 0.23; I/II = 0.21			
	STN [g · kg^{-1}]							
T. sphaerococcum	*a* 0.94 a ± 0.04[1]	*b* 0.67 b ± 0.04	*b* 0.68 b ± 0.03	0.76 B ± 0.14	*b* 1.05 a ± 0.04	*a* 1.16 a ± 0.01	*a* 1.19 b ± 0.02	1.14 A ± 0.07
T. persicum	*b* 0.81 b ± 0.04	*a* 1.10 a ± 0.07	*c* 0.75 a ± 0.03	0.89 A ± 0.16	*c* 0.87 b ± 0.02	*b* 1.05 b ± 0.04	*a* 1.24 a ± 0.05	1.05 B ± 0.16
mean	0.88 *A* ± 0.08	0.88 *A* ± 0.24	0.72 *B* ± 0.05	0.82 ± 0.16	0.96 *C* ± 0.10	1.10 *B* ± 0.07	1.22 *A* ± 0.05	1.09 ± 0.13
$HSD_{0.05}$	I = 0.002; II = 0.003; II/I = 0.005; I/II = 0.003				I = 0.002; II = 0.002; II/I = 0.003; I/II = 0.003			
	SNO_3^- [mg · kg^{-1}]							
T. sphaerococcum	11.83 a ± 0.450	11.00 ± 0.325	10.03 ± 0.388	10.95 B ± 0.807	20.76 ± 0.991	18.76 ± 0.466	17.11 ± 0.642	19.21 A ± 1.610
T. persicum	12.58 ± 0.807	11.23 ± 0.923	10.53 ±0.887	11.46 A ± 0.923	18.43 ± 1.610	19.33 ± 1.691	18.87 ± 1.678	18.88 B ± 1.690
mean	12.20 *A* ± 0.45	11.14 *B* ± 0.325	10.28 *C* ± 0.388	11.20 ± 0.887	19.60 *A* ± 0.991	19.04 *B* ± 0.466	18.50 *C* ± 0.642	19.05 ± 1.678
$HSD_{0.05}$	I= 0.43; II = 0.40; II/I = ns[2]; I/II -= ns				I = 0.75; II = 0.57; II/I = ns; I/II = ns			

OF – organic farming; CF – conventional farming; [1]SD – standard deviation; [2]ns – not statistically significant; SOC – soil organic carbon; STN – soil total nitrogen; SNO_3^- – soil nitrate nitrogen.

Different capital letters indicate a comparison within the I factor (vertically), and capital italic letters indicate a comparison within the II factor (horizontally).

Different small italic letters (horizontally) indicate a comparison within interaction II/I, and small letters (vertically) indicate a comparison within the interaction I/II.

Values followed by the same small or capital letter within each column are not significantly different at $p = 0.05$; HSD – honestly significant difference

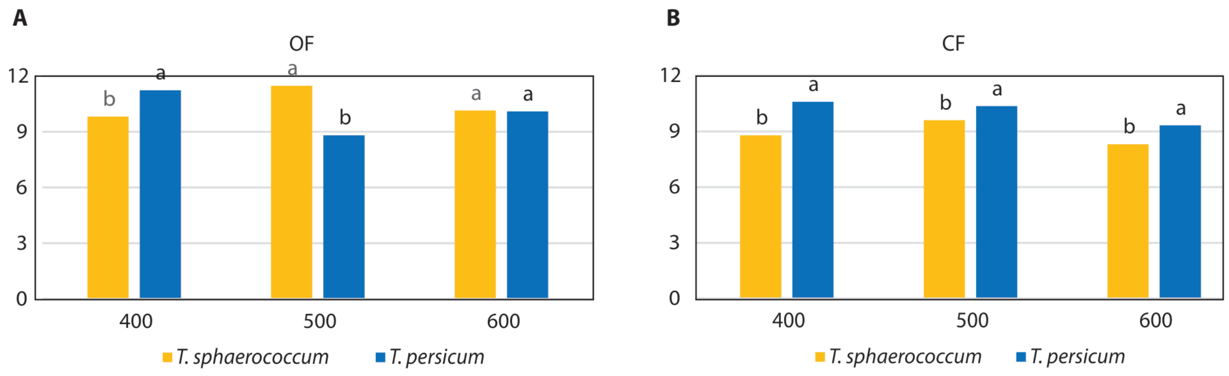

Fig. 1. Values of SOC-STN ratio in soil conventional farming (A) and in soil organic farming (B)

(Fig. 1B). This indicated greater accumulation of carbon than nitrogen under organic farming (Pecio and Jarosz 2016). In arable soils, the SOC/STN ratio averages 10. A narrow SOC/STN ratio indicates a high degree of humification of organic matter, but also its overly rapid mineralization in the soil (Lemanowicz *et al.* 2024). The lower SOC/STN ratios under CF soil are crucial for soil microorganisms to avail themselves of SOC and STN, which undoubtedly promotes increased mineralization of organic substances and the release of mineral forms of N (Guo *et al.* 2018). It causes the release of larger amounts of nutrients into the soil and thus affects soil fertility. Studies by Duan *et al.* (2021) showed that SOC (soil organic carbon) and SOC/TN values increased with increasing plant density up to 800 plants m^{-2}, and then decreased with increasing plant density up to 1600 and 3200 plants · m^{-2}. This was probably related to the fact that at low density, wheat bound less energy through photosynthesis per unit area, which reduced the transport of organic matter to the soil. At higher density, there was strong intraspecific competition, which led to a decrease in SOC and thus SOC/TN. This was confirmed by subsequent studies by Duan *et al.* (2024).

Nitrate content in the soil depends primarily on soil type and the applied form and dose of N fertilizer (Yan *et al.* 2016). The present studies showed that the SNO_3^- content in the soil significantly depended on the wheat variety and sowing density, regardless of the farming system. The highest SNO_3^- content was found in the soil in which *T. sphaerococcum* was grown (10.95 mg · kg^{-1} – OF; 19.21 mg · kg^{-1} – CF) and at a sowing density of 400 grains · m^{-2}, 12.20 mg · kg^{-1} (OF) and 19.60 mg · kg^{-1} (CF), respectively (Table 2). According to Anjana and Iqbal (2007), Cui *et al.* (2008), García-Ruiz *et al.* (2019) and Sahoo *et al.* (2022), soil nitrate content depended not only on the farming system but also on the wheat genotype and cultivation time (plant development stage). However, a single application of N at the beginning of the crop cycle effectively controls nitrate accumulation in the soil as well as in the plant, and, importantly, nitrate concentration decreases when the plants reach commercial size (Anjana and Iqbal 2007; Dai *et al.* 2013; Zhang *et al.* 2015; Xiao *et al.* 2022; Zhang *et al.* 2021). The optimization of plant seeding density is an important agrotechnical procedure that improves nitrogen use efficiency. Increasing plant density decreases nitrate content in deeper soil layers and may at the same time stimulate N uptake from these layers of the soil profile (Zhang *et al.* 2015).

Analysis of variance showed that sowing density had a significant effect on PRO activity in the OF soil (Table 3). Much of the highest PRO activity (32.62 mg TYR · kg^{-1} · h^{-1}) was obtained at a density of 500 grains · m^{-2}. The interaction of applied factors significantly shaped PRO activity in the soil. Research by Schoebitz *et al.* (2020) did not show any significant differences in the activities of enzymes (urease, protease, dehydrogenases, β-gluosidase) at different plant densities. The exception was phosphatase, which was 152% higher in treatments with high plant density. Under CF, PRO activity was significantly influenced by wheat variety and sowing density. The OF soil had 109% lower PRO activity than CF. This was related to the OC content in the soil. However, Marinari *et al.* (2006) noticed that the activity of proteases was higher in the soil from plots managed organically. Similar results have been presented by Kwiatkowski *et al.* (2020). The results of correlation analysis showed a positive relationship between PRO activity and SOC and STN content in both CF and OF, respectively: $r = 0.66$; $r = 0.91$ for CF and $r = 0.52$; $r = 0.67$ for OF (Fig. 2A and Fig. 2B).

In the present studies, the content of WTN in the aboveground parts of spring wheat was higher in that collected from CF than from OF (Table 4). According to Mäder *et al.* (2007), conventional farming employs much greater quantities of mineral fertilizers (including nitrogen) than organic farming, resulting in greater nitrogen content in CF plants. The chemical composition of cereals varies greatly both among and within species and depends on the environmental

Table 3. Activity of proteases (PRO) (mg TYR · kg^{-1} · h^{-1}) in soil

Wheat species I factor	OF				CF			
	Sowing density [grains · m^{-2}] – II factor							
	400	500	600	mean	400	500	600	mean
Triticum sphaerococcum	*a*43.29 a ± 0.361[1]	*b*30.07 b ± 0.812	*b*26.61 a ± 0.302	33.32 ± 0.425	57.28 ± 0.862	68.93 ± 0.969	7.71 ± 1.158	67.97 A ± 0.957
T. persicum	*b*22.47 b ± 0.802	*a*39.16 a ± 0.611	*b*27.12 a ± 0.713	29.58 ± 0.368	51.04 ± 0.725	64.21 ± 0.788	75.70 ± 1.057	63.65 B ± 0.917
mean	32.88 *B* ± 14.72	34.62 *A* ± 11.80	26.86 *C* ± 0.36	31.45 ± 2.64	54.16 *C* ± 4.41	66.57 *B* ± 3.34	76.71 *A* ± 1.42	65.81 ± 3.05
$HSD_{0.05}$	I = ns[2]; II = 1.125; II/I = 8.60; I/II = 1.59				I = 0.275; II = 2.68; II/I = ns; I/II = ns			

OF – organic farming; CF – conventional farming; [1]SD – standard deviation; [2]ns – not statistically significant

Different capital letters indicate a comparison within the I factor (vertically), and capital italic letters indicate a comparison within the II factor (horizontally).

Different small italic letters (horizontally) indicate a comparison within interaction II/I, and small letters (vertically) indicate a comparison within the interaction I/II.

Values followed by the same small or capital letter within each column are not significantly different at $p = 0.05$; HSD – honestly significant difference

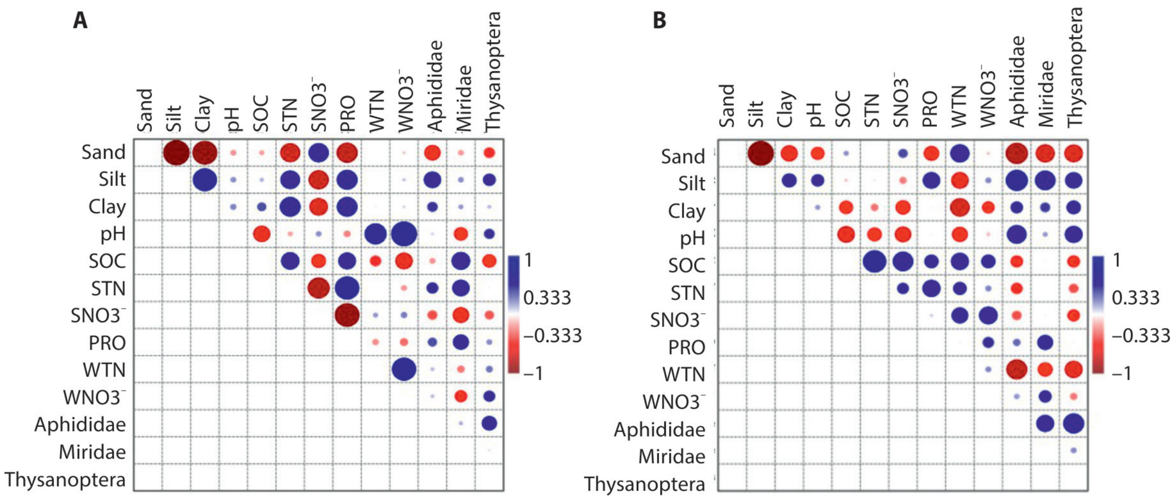

Fig. 2. Correlogram of the studied variables in conventional farming (A) and organic farming (B)

conditions of the soil, which include the effect of fertilization (Feng and Dietze 2013; Gebremariam *et al.* 2014). As nitrogen fertilizer application increases, leaf N content initially increases and then stabilizes (Cocco *et al.* 2015). Nitrogen fertilizers improve the photosynthetic capacity of plants (Khan *et al.* 2022; Yu *et al.* 2022; Simkin *et al.* 2020). The influence that a plant's physiological traits have on photosynthetic parameters varies, and it depends on the species, functional type, and taxonomic scale of the plant. The intraspecies variability of photosynthetic parameters during the growing season can be attributed to seasonal changes in leaf characteristics, especially nitrogen and chlorophyll content (Feng and Dietze 2013). The tested wheat varieties differed significantly in WTN content only under CF. Similar conclusions have been presented by Atia and Ragab (2013), who also showed that the uptake of nitrogen by plants is dosage-dependent. Regardless of variety, the use of higher N doses increased N content in plants. Moreover, Mäder *et al.* (2007) and Atia and Ragab (2013) found that the varieties that contained the most N were characterized by the highest uptake of N from the soil. The present study confirmed this relationship. The *Triticum sphaerococcum* variety showed a higher WTN content than the *Triticum persicum* variety, under both OF (23.64 mg · kg^{-1}) and CF (35.64 mg · kg^{-1}). Analysis of variance also showed sowing density to have a significant effect on WTN content under each farming system. The planting density that yielded the highest average nitrogen content under each system was 500 grains · m^{-2} for OF (24.19 mg · kg^{-1}) and 400 grains · m^{-2} for CF (37.61 mg · kg^{-1}) (Table 4). Jaswinder *et al.* (2019) showed that reduced sowing density reduced competition for nutrients in the soil. This resulted in increased nutrient uptake by plants. The N content in plants depends mostly on its form and availability in the soil (Doltra *et al.* 2011). This was confirmed by the

Table 4. Nitrogen compound contents in spring wheat plants

Wheat species – I factor	OF				CF			
	Sowing density [grains · m^{-2}] – II factor							
	400	500	600	mean	400	500	600	mean
	WTN [g · kg^{-1} d.m.]							
Triticum sphaerococcum	24.25 ± 1.48[1]	23.65 ± 1.30	22.59 ± 1.76	23.64A ± 1.03	37.05ab ± 3.13	35.86b ± 3.18	33.71c ± 2.21	35.64A ± 1.89
T. persicum	22.25 ± 1.03	24.74 ± 1.01	23.49 ± 1.62	23.50B ±1.01	38.17a ± 1.89	32.92c ± 1.15	35.45b ± 3.09	35.54B ± 1.15
mean	23.25*AB* ± 1.48	24.19*A* ± 1.30	23.22*B* ± 1.76	23.57 ± 1.62	37.61*A* ± 3.13	34.39*B* ± 3.18	34.78*B* ± 2.21	35.59 ± 3.09
$HSD_{0.05}$	I = 0.12; II = 0.90; II/I = ns[2]; I/II = ns				I = 0.09; II = 0.63; II/I = 0.89; I/II = 0.63			
	WNO_3^- (mg · kg^{-1} d.m.)							
T. sphaerococcum	1276.6a ±11.97	1258.7bc ± 10.56	1240.0c ± 10.72	1279.8A ± 24.55	1302.6b ± 14.58	1278.4d ± 26.06	1273.0e ± 28.39	1291.0A ± 7.60
T. persicum	1262.2b ±24.55	1254.7bc ± 9.46	1258.3bc ± 18.59	1258.4B ± 9.456	1306.2a ± 7.60	1263.2f ± 19.40	1284.7c ± 26.25	1284.8B ± 19.40
mean	1269.4*B* ±11.97	1256.7*C* ± 10.56	1281.21*A* ± 10.72	1269.1 ± 18.59	1304.4*A* ± 14.58	1270.8C ± 26.06	1288.8B ± 28.39	1288.0 ± 26.25
$HSD_{0.05}$	I = 6.50; II = 7.31; II/I = 10.34; I/II = 9.32				I = 3.09; II = 1.98; II/I = 2.80; I/II = 3.55			

WTN – total nitrogen in wheat; WNO_3^- – nitrate nitrogen in wheat; OF – organic farming; CF – conventional farming; [1]SD – standard deviation; [2]ns – not statistically significant

Different capital letters indicate a comparison within the I factor (vertically), and capital italic letters indicate a comparison within the II factor (horizontally).

Different small italic letters (horizontally) indicate a comparison within interaction II/I, and small letters (vertically) indicate a comparison within the interaction I/II.

Values followed by the same small or capital letter within each column are not significantly different at $p = 0.05$; HSD – honestly significant difference

correlation coefficient between the SNO_3^- content in the soil and WTN ($r = 0.62$) (Fig. 2A).

Plants use the absorbed nitrogen to synthesize amino acids, proteins, and other complex nitrogen compounds (Singh and Sood 2017). It is possible that if nitrates are assimilated by plants to a significantly lesser extent than they are taken up, then they accumulate excessively in tissues, which is detrimental to plant growth. In crops other than legumes, nitrate concentration is higher in the aboveground parts of plants than in the underground parts (Anjana and Iqbal 2007). In the present experiment, the varieties differed significantly in the content of nitrates in plants, under both organic and conventional systems (Table 4). According to Harrison *et al.* (2004) and Anjana and Iqbal (2007), the nitrate content in plants depends on the species, the cultivar of the same species, and even differences in ploidy within a genotype. This may be due to differences in the enzymatic pathways of nitrogen metabolism (nitrate reductase/nitrite reductase), the uptake rates for nitrates and other elements necessary for enzyme activity, or the production of required electron donors in the assimilation pathway. It should be borne in mind that the control of the uptake and accumulation of nitrates in a plant may be subject to genetic variability regardless of the plant's needs. This variability allows for a regulatory control mechanism that is adaptive to the availability of nitrate in the soil (Anjana and Iqbal 2007). It was shown that increasing sowing density generally decreases nitrate content in the aboveground parts of the tested spring wheat varieties (Table 4). However, NO_3^- content in the soil decreases in direct proportion to an increase in plant density (Table 2). This may be due to the plant's proportion of root mass to aboveground mass, which increases with sowing density (Hecht *et al.* 2016). At a sowing density of 500 grains · m^{-2}, plants had the lowest WNO_3^- content (1,256.7 mg · kg^{-1} for OF; 1,270.8 mg · kg^{-1} for CF). Moreover, genotype and sowing density were shown to interact in determining the WNO_3^-content in the aboveground parts of wheat. The content of WNO_3^- was the smallest at a sowing density of 600 grains · m^{-2} *Triticum sphaerococcum Perc.* and 500 grains · m^{-2} *Triticum persicum*, for both OF and CF.

The correlation analysis showed a positive relationship between WNO_3^- in the plant and the occurrence of Miridae ($r = 0.462$ for OF) (Fig. 2B). An understanding of the relationship between plant nutrition and pest reproductive potential is important for pest control in modern agroecosystems (Zarasvanda *et al.* 2013). The major determinants of plant susceptibility to pests include stage of plant development, nutrition, and type of agrotechnical treatments applied (Rouhani *et al.* 2012; Huber *et al.* 2012). According to Huber *et al.* (2012), well-nourished plants are less susceptible to pest attacks. This relationship was also confirmed by the present study (r = -0.460 for CF) (Fig. 2A). However, it was noted that this relationship was observed in wheat under conventional farming only. According to Huber *et al.* (2012), plant susceptibility to pests depends on the type of nutrients in the plant and thus on the farming system. Furthermore, the susceptibility of plants to pests also depends on the plant species and the type or virulence of the pathogen. Among the analyzed insects with piercing-sucking mouthparts, the most abundant were thrips. These insects numbered 33.92 ind. 24 · m^{-2} for OF and 20.96 ind. 24 · m^{-2} for CF (Table 5). The second most abundant were Aphididae (27.92 ind. · 24 m^{-2} for OF; 12.35 ind. · 24 m^{-2} for CF). The least abundant insects with such mouthparts were Miridae (2.48 ind. · 24 m^{-2} for OF; 0.77 ind. · 24 m^{-2} for CF). Insects were more abundant under OF than CF. Similar total insect numbers were noted by Lemanowicz *et al.* (2020). In spring wheat plots (irrespective of variety), the average number of insects caught at the beginning of the flowering stage was 167 for OF, compared to only 91.8 for CF. According to Kaniuczak and Bereś (2011), the number of insects on cereals grown organically is greater because the methods for regulating the number of phytophages feeding on them are limited. In the present study, more aphids (Aphididae) and thrips (Thysanoptera) occurred on round-grain wheat than on Persian wheat. Regardless of plant density, hemimetabolous insects preferred feeding on round wheat plants rather than Persian wheat plants; only for Miridae – and only under CF – did the statistical analysis reveal no significant

Table 5. Density of pests in spring wheat plants (ind. · 24 m^{-2})

Wheat species I factor	OF				CF			
	Sowing density [grains · m^{-2}] – II factor							
	400	500	600	mean	400	500	600	mean
	Aphididae							
Triticum sphaerococcum	32.88 ± 0.479[1]	37.75 ± 1.190	33.75 ± 1.190	34.79 A ± 2.398	*a*15.13 a ± 0.854	*b*9.50 b ± 0.408	*a*14.75 a ± 0.645	13.13 A ± 2.748
T. persicum	19.63 ± 1.109	22.25 ± 1.190	21.25 ± 0.957	21.04 B ± 1.499	*c* 9.75 b ± 0.289	*b*11.63 a ± 0.854	*a*13.38 b ± 0.946	11.58 B ± 1.690
mean	26.25 *B* ± 7.126	30.00 *A* ± 8.358	27.50 *B* ± 6.756	27.92 ± 7.290	12.44 *B* ± 2.933	10.56 *C* ± 1.293	14.06 *A* ± 1.050	12.35 ± 2.366
$HSD_{0.05}$	I = 0.795; II = 1.535; II/I = ns[2]; I/II = ns				I = 0.697; II = 0.839; II/I = 1.186; I/II = 0.903			
	Miridae							
T. Sphaerococcum	*a* 7.25 a ± 0.289	*b* 2.63 a ± 0.479	*b* 3.37 a ± 1.109	4.42 A ± 2.214	*a* 0.50 a ± 0.408	*a* 0.87 a ± 0.479	*a* 0.87 a ± 0.250	0.75 ± 0.399
T. persicum	*a* 0.25 b ± 0.289	*a* 0.37 b ± 0.479	*a* 1.00 b ± 0.408	0.54 B ± 0.498	*b* 0.00 b ± 0.000	*a* 1.25 a ± 0.289	*a* 1.12 a ± 0.479	0.79 ± 0.656
mean	3.75 *A* ± 3.751	1.50 *C* ± 1.282	2.19 *B* ± 1.487	2.48 ± 2.526	0.25 *B* ± 0.378	1.06 *A* ± 0.417	1.00 *A* ± 0.378	0.77 ± 0.531
$HSD_{0.05}$	I = 1.153; II = 0.533; II/I = 0.753; I/II = 1.051				I = ns; II = 0.351; II/I = 0.497; I/II = 0.429			
	Thysanoptera							
T. Sphaerococcum	*b*28.63 a ± 1.109	*a*49.00 a ± 0.913	*a*49.63 a ± 1.436	42.42 A ± 10.244	*b*22.63 a ± 1.031	*c*17.50 b ± 0.816	*a*29.00 a ± 1.414	23.04 A ± 5.016
T. persicum	*b*23.00 b ± 0.408	*a*35.00 b ± 1.000	*c*18.25 b ± 0.957	25.42 B ± 7.400	*a*19.25 b ± 0.866	*a*21.00 a ± 1.080	*b*16.38 b ± 1.436	18.88 B ± 2.247
mean	25.81 *C* ± 3.105	42.00 *A* ± 7.536	33.94 *B* ± 16.809	33.92 ± 12.320	20.94 *B* ± 2.008	19.25 *C* ± 2.070	22.69 *A* ± 6.876	20.96 ± 4.356
$HSD_{0.05}$	I = 1.125; II = 1.183; II/I = 1.674; I/II = 1.334				I = 1.281; II = 1.342; II/I = 1.898; I/II = 1.515			

[1] OF – organic farming; CF – conventional farming; [1]SD – standard deviation; [2]ns – not statistically significant

Different capital letters indicate a comparison within the I factor (vertically), and capital italic letters indicate a comparison within the II factor (horizontally).

Different small italic letters (horizontally) indicate a comparison within interaction II/I, and small letters (vertically) indicate a comparison within the interaction I/II.

Values followed by the same small or capital letter within each column are not significantly different at p = 0.05; HSD – honestly significant difference

difference in the abundance of these insects. As reported by Lemanowicz *et al.* (2020), in wheat, under both OF and CF, significantly more insects were netted from *T. sphaerococcum* than from *T. persicum* (respectively: 208.3 and 125.8 ind. · 24 m^{-2} for OF; 93 and 90 ind. · 24 m^{-2} for CF). Similarly, Szczepanek *et al.* (2020) noted that *Oulema* spp. larvae differed as to which wheat species they preferred as food, with *T. sphaerococcum* being much more attractive than *T. persicum*. Analysis of the effect of plant density on the abundance of examined insects showed that, for OF, the species were netted in the greatest numbers as follows: Thysanoptera and Aphididae at a sowing density of 500 grains · m^{-2} and Miridae at a lower density, i.e., 400 grains · m^{-2} (respectively: 42.00, 30.00 and 3.75 ind. 24 m^{-2}). Meanwhile, for CF, Thysanoptera and Aphididae were the most numerous at a sowing density of 600 grains · m^{-2} and Miridae at a slightly lower density of 500 grains · m^{-2} (respectively, 22.69, 14.06 and 1.06 ind. · 24 m^{-2}). Lemanowicz *et al.* (2020) report that, for organically grown spring wheat, Insecta prefer higher densities (500 and 600 grains · m^{-2}), whereas for CP spring wheat plants they prefer an average density of 500 grains · m^{-2}.

Significant correlations between insect number and WNO_3^- were found more frequently for OF wheat (Fig. 2B). The abundance of all insect groups was strongly negatively correlated with nitrate N content in wheat plants (*T. sphaerococcum*) as follows: Aphididae (r = –0.785), for Miridae (r = –0.552) and for Thysanoptera (r = –0.673).

Conclusions

Soil nitrogen status has a wide range of effects on plant–pest interactions, increasing or decreasing the attractiveness of plants as a food.

The study was conducted to determine the effect that growing two spring wheat varieties (*Triticum sphaerococcum* and *Triticum persicum*) at different plant densities under organic and conventional farming has on the number of hemimetabolous insects determined by the nitrogen content in plants. These relationships were shown against the background of selected chemical and biochemical properties of the soil.

The experimental factors used were, i.e., spring wheat variety and sowing density which determined the content of organic carbon and total nitrogen in the soil. However, the tested soil was characterized by low soil organic carbon content. This indicated that the use of additional treatments to enrich the soil with organic matter is recommended. Protease activity was positively correlated with organic carbon and total nitrogen content in the soil, which indicated that this hydrolytic enzyme had a role in organic matter cycling.

Insects with piercing-sucking mouthparts decidedly prefer cereal plants grown under organic farming conditions over conventional farming. However, these pests do not occur in such abundance that threaten the growth and development of the host plant. Hemimetabolous insects are much less numerous on Persian wheat than on round grain wheat. In the case of conventional farming, a negative relationship was found between the content of nitrate nitrogen in spring wheat and the occurrence of Miridae. A positive relationship was found between the content of total nitrogen in the soil and the content in spring wheat grown in organic farming. This relationship negatively affected the feeding of Thysanoptera, Aphididae and Miridae.

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