



## Causal relationships between wheat productivity and biological activity and soil nitrogen content

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**Abstract.** Coverage of the components of causal dependencies of crop productivity mediated by N-, C-chemical, biological indicators of soil (sizes of pools of relevant available forms, enzymatic activities) in the context of theoretical aspects of resource turnover in it is potentially significant for the design of systems of evaluation criteria for soil health using machine learning (ML) models. The purpose of this study was to find statistically valid and meaningful coordination and causal relationships of grain weight per sown area with available forms of N, C (inorganic and easily hydrolysed N – NIN, NEH; C of labile humus – CLH), in situ proteolytic PA, in vitro urease UA activity in association with the concepts of the turnover of these organogens in the soil under winter wheat under prototypical environmentally friendly fertiliser systems. The

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following methods were employed: field, in situ, laboratory-analytical chemical and biochemical, morphometric, statistical, ML-modelling. With a high probability, the increase in GM was caused by an additive increase in NIN, NEH, and a simultaneous decrease in in situ PA, in a way that mediated the effect of in situ PA on GM by the listed forms of N. Statistically less valid was the mediation of the effect of the ratio of NEH to NIN on GM through in situ PA with possibly mutually complementary predispositions of N forms, which, however, provided the basis for substantiating the simultaneous mutually opposite determination of GM by these N forms and in situ PA. It is plausible that CLH and in vitro UA were not involved in the development of GM under these experimental conditions, but it is possible that the latter of these variables may have a more significant effect on the variation of the former than vice versa. Studies in this vein will develop a discourse of theoretical predictions regarding the improvement of the structure of soil quality indices towards revealing aspects of the laws of promoting the balance between the characteristics of agro-ecosystem services, considering the chemical and biochemical properties of N and C turnover in the soil, and crop bioproductivity necessary for the development of sustainable agriculture

**Keywords:** available forms of nitrogen in soil; labile humus carbon; proteolytic and urease activity of soil; winter wheat grain weight; exploratory factor analysis; systems of simultaneous equations; causal determinants

## INTRODUCTION

Nitrogen (N) is a key element for primary production (PP) and soil organic matter (SOM) stabilisation in agroecosystems. Microbial activity (MA) of the soil, especially the enzymatic activity (EA) of N-mineralising enzymes (NMEs), is essential for the release of bioavailable N (BAN) from soil organic matter (OM) (Daunoras *et al.*, 2024; Wang *et al.*, 2024). According to N. Fierer *et al.* (2021), EA is a significant indicator of soil quality and health, characterising its ecological functions and ecosystem services to support bioproductivity. As noted by J. Lehmann *et al.* (2020), the analysis of NME variations is significant for assessing soil health and understanding crop production. However, a series of obstacles, such as interactions between soil components, asynchrony of N (Zhu *et al.*, 2025), etc., complicate the interpretation of changes in EA, N-forms, which also complicates the identification of the relative significance and causality of EA(NME) – BAN – bioproductivity.

Understanding the complex causal relationships between agro-technological interventions, soil characteristics, and crop productivity is key to the development of sustainable agriculture. The literature has identified two principal ways in which such links can be established. The first (*i*) involves the direct or indirect impact of agricultural technologies on soil EA, which leads to changes in SOM and nutrient release, which ultimately affect yields (Hassan *et al.*, 2022). The second (*ii*) focuses on the modification of substrates (S)/intermediates/products by agricultural technologies, which causes changes in EA, which subsequently affect nutrient accumulation, with a possible positive feedback effect and impact on yield (Tian *et al.*, 2020). The following literature review details studies that confirmed and extended these general models by considering the impact of individual factors and processes on EA, N-forms, and the interdependencies between them.

In this context, it is essential to note that S availability and nutrient limitation (LIM) are crucial factors in the regulation of NME and C- and P-metabolism

enzymes. The principles of soil ecology and biochemistry expand the understanding of the interdependencies between EA and S, nutrients-LIM. Specifically, X. Tan *et al.* (2021) argued that eco-EA (EEA) of C, N, P cycles, and EE-stoichiometry (EES) depend on the state, nutrient-stoichiometry, relative LIM/availability of microbial resources, soil microbial properties, and environmental conditions. According to the theory of resource allocation, microorganisms can release exoenzymes (increase in EEA) in response to C-, N-, P-LIM to produce LIM nutrients (negative nutrient-efficiency relationships – EEA). However, there may also be an increase in EEA with the addition of S or no clear relationship. The significance of regulating NME and other enzymes is also conditioned by the fact that the uptake of bioavailable N (BAN) by crops is mainly from the soil, particularly from the turnover of SOM and soil organic nitrogen (SON) (Yan *et al.*, 2020). Therefore, the values of the balance sizes of the  $N_{IN}$ ,  $N_{EH}$ ,  $C_{LH}$  pools, potentially affecting GM, can be predetermined (regulated) by EA (NME). At the same time, these pools may well be involved in the regulation of isPA, ivUA (feedback loop).

Admittedly, earlier studies by Q. Ma *et al.* (2020), B. Adamczyk (2021), revealed that soil PA is a crucial LIM factor in mineralisation and N availability, although L. Greenfield *et al.* (2020) suggested that the rate of protein supply may have a greater influence on differences in N turnover. PA regulation is a complex process that is influenced by a variety of abiotic and biotic factors, such as NPP,  $NO_3^-$  concentration, C:N, and soil height and depth. T. Jesmin *et al.* (2021) noted a  $NH_4^+/NO_3^-$ -repressive effect on PA, but suggested a stimulating effect of organic C, N-compounds of plant residues on this NME. Since the SON dynamics, which is determined by SOM and MBC via MA (EA), is vital for the accumulation of mineralised S, it is natural that MBC and initial SOM directly and indirectly through PA and glutaminase affect the SON dynamics.

The mutual regulation between nutrient pools and UA is less understood. Overall, nutrient availability ( $\text{NH}_4^+$ , SOM, SON), C:N are crucial regulators of UA, where  $\text{NH}_4^+$ -sensitivity may vary between distinct groups of bacteria (Chettri *et al.*, 2021). K. Ampong *et al.* (2022) also showed that the effect of humic acids on soil UA can be different and depends on their type, origin, and can be modulated by MB, C:N, nitrification/denitrification. UA can be induced by the presence of N and OM but inhibited by high concentrations of  $\text{NH}_4^+$  as the only N source. Further mineralisation processes are also subject to complex regulation involving S/intermediates/reaction products. The key factors controlling the mineralisation rate are the quantity and quality of detritus and natural fertilisers. Furthermore, C:N in SOM plays a significant role in determining the mineralisation/immobilisation balance (Dalias & Christou, 2022). However, Y. Cao *et al.* (2021) argued that the key role in regulating the response of microbial N immobilisation to OC addition belongs to the characteristics of its chemical quality, which is determined by the ratios of labile, intermediate, and recalcitrant pools.

Nitrification, as one of the final stages of complete N-mineralisation, is also characterised by complex regulation.  $\text{NH}_4^+$  is S for nitrifying bacteria and therefore accelerates nitrification. On the other hand, high C:N can lead to competition for N among microorganisms, decelerating nitrification. The  $\text{NH}_4^+$  requirement of plants can also affect the rate of nitrification, as plants are the primary consumers of mineral N.  $\text{O}_2$  is essential for aerobic nitrifying bacteria and therefore stimulates nitrification while inhibiting anaerobic denitrification. Analysing the relationships between denitrification and readily available SOC (energy source),  $\text{O}_2$  and  $\text{NO}_3^-$ /mineral N in soil, B. Pan *et al.* (2022) found that the latter contributed to increased  $\text{N}_2$  emissions. The ratio of  $\text{N}_2\text{O}/(\text{N}_2\text{O} + \text{N}_2)$  increased with increasing soil  $\text{O}_2$  but decreased with SOC, C:N, and soil pH. Therewith, G.P. Robertson and P.M. Groffman (2024) argued that with sufficient SOC and  $\text{O}_2$  deficiency,  $\text{NO}_3^-$  concentration can become a LIM factor in denitrification. Global studies (structure equation modelling, SEM) conducted by Z. Li *et al.* (2020) showed that total nitrogen (TN) and microbial nitrogen (MBN) are positively correlated with nitrification, which is also related to SOM content. An increase in C-LIM (especially microbial) and a decrease in N-LIM (leading to an increase in SON) can stimulate both N-mineralisation and nitrification.

Thus, there is a complex network of interactions/interregulations and causality between biological and chemical factors that control N cycling in soil, including nutrient availability, EA, OM. Mineralisation, nitrification/denitrification are regulated by various biotic and abiotic factors, including C:N,  $\text{O}_2$ , MB. Understanding these relationships is key to assessing the influence of agroecosystems on soil productivity and stability. The purpose of this study was to determine the stochastic

(inter)coordination and causal relationships between the soil traits  $\text{N}_{\text{IN}}$ ,  $\text{N}_{\text{EH}}$ ,  $\text{C}_{\text{LH}}$ , isPA, ivUA and GM of winter wheat under ESFS conditions.

## MATERIALS AND METHODS

The study described in this publication was conducted in 2021-2022 on grey forest surface-gleyed sandy-loam soil under winter wheat (*Triticum aestivum* L.) of the Benefis variety (stationary experiment to investigate the scientific basis of productivity management of short-term crop rotations in the Carpathian region; Institute of Agriculture of the Carpathian Region of the National Academy of Agrarian Sciences of Ukraine). The experimental microplots (area = 1 m<sup>2</sup>; replication = 3) were arranged systematically; the distance between them was 0.5 m. Before setting up the experiment, the key physicochemical and agrochemical parameters of the soil were determined (depth 0–30 cm):  $\text{pH}_{\text{KCl}}$  = 4.78-4.92, hydrolytic acidity (according to Kappen) 23.83-24.61 mg-eq kg<sup>-1</sup> of the soil, the content of the easily hydrolysed nitrogen ( $\text{N}_{\text{EH}}$ ; according to Kornfield) 86.22-91.32 mg kg<sup>-1</sup> of the soil, available phosphorus, available potassium (according to Kirsanov; extraction with 0.2 M HCl) – 105.11-113.04 mg kg<sup>-1</sup> of the soil and 84.03-90.17 mg kg<sup>-1</sup> of the soil, respectively, the carbon (C) content of total humus (according to Tyurin) – 1.91-1.96%. All experimental variants (ecologically safe technologies) were divided into 2 groups (each with its own control), depending on the applied precursor: i) variants of group A (A-1–A-7); the precursor was pea (*Pisum sativum* L.); ii) variants of group B (B-1–B-7); the precursor was fodder bean (*Vicia faba* L. var. major Harz). The layout of the typical agrotechnical and ecologically safe factors in the listed variants 1-7 of groups A, B was the same, and was as follows: A-1) control (no mineral or organic fertilisers or ecologically safe factors were used; the precursor was pea); A-2) FPS (field pea straw); A-3) FPS + RMF (recommended rate of mineral fertilisers –  $\text{N}_{90}\text{P}_{60}\text{K}_{60}$ ); A-4) FPS + RMF + BS (biostimulant “Millerplex”); A-5) FPS + RMF + BS + HF (humic fertiliser “BlackJak”); A-6) FPS + RMF + CF (chelated fertiliser of the “Rozalik” range); A-7) FPS + HMF (high rate of mineral fertilisers –  $\text{N}_{150}\text{P}_{120}\text{K}_{120}$ ) + HF; B-1) control (mineral or organic fertilisers or the ecologically safe factors were absent; precursor – fodder beans); B-2) FBS (fodder beans straw); B-3) FBS + RMF (recommended rate of mineral fertilisers –  $\text{N}_{90}\text{P}_{60}\text{K}_{60}$ ); B-4) FBS + RMF + BS (biostimulant “Millerplex”); B-5) FBS + RMF + BS + HF (humic fertiliser “BlackJak”); B-6) FBS + RMF + CF (chelated fertiliser of the “Rozalik” range); B-7) FBS + HMF (high rate of mineral fertilisers –  $\text{N}_{150}\text{P}_{120}\text{K}_{120}$ ) + HF. A brief description, methods, and time of application of the commercial preparations used in the study – components of the ecologically safe factors (bioeffectors), are presented below.

The biostimulant (BS) “Millerplex”. Treatment of plants twice during the spring-summer vegetation in

the phase of spring tillering, tubing at 0.5 L ha<sup>-1</sup>. Contains natural cytokinins from 3 plant sources, which have enhancing properties for the hormonal regulation of growth of winter cereals at the cellular level, particularly under stressful conditions. Composition of the preparation: N amide – 3.0%, P<sub>2</sub>O<sub>5</sub> – 3%, K<sub>2</sub>O – 30%, algae extract (*Ascophyllum nodosum*); pH – 6.0-6.5; additionally, amino acids, specific carbohydrates (improve the immune system of plants), trace elements (Cu, Fe, Mn, Mg, Zn, B, Co) in chelated form. Built-in adjuvant system for maximum penetration and translocation of biological components into plants. The humic fertiliser (HF) “BlackJak”. Spraying on the surface of the root zone twice during the spring-summer growing season in the phases of spring tillering and tubing at 1.0 L ha<sup>-1</sup>. Has a prominent biostimulant effectiveness; apart from humic and fulvic acids, it contains ulmic acids and humin (the most active in plants). Composition: humic acids – 19-21%, fulvic acids – 3-5%, total organic matter – 27-30%; pH – 3.5-5.0. The product is effective both for plants and is most useful in the soil. The chelated fertiliser (CF) of the “Rozalik” range (Zn, P, N, S). Treatment of plants twice during the spring-summer growing season in the phases of spring tillering, tubing at 2.0 L ha<sup>-1</sup>. Composition: N amide – 3%, P<sub>2</sub>O<sub>5</sub> – 19%, SO<sub>3</sub> – 5.3%, Zn in the Zn-EDTA form – 5.9%.

The dynamics of synoptic conditions during the autumn of 2020 and the growing seasons of 2021-2022 was characterised by a combination of the following properties: *i*) the winter and spring-summer intervals of 2020-2021 and 2021-2022 differed in terms of precipitation (PI); in the winter-summer period of 2021, PI was 473.6 mm, while in 2022 it was 318.3 mm (average long-term PI values were 458.0 mm); more than half of these precipitations occurred in the winter-spring interval; *ii*) in 2022 there was a slight lack of moisture during March, May-June, which, however, did not affect biological processes in the soil, plant development, and their final productivity; *iii*) during the entire time interval of the study, the temperature regime of the soil and atmospheric air was predominantly favourable for biological processes in the substrate, for the growth and development of winter wheat; overall, the average monthly temperatures were comparable during the spring and summer periods of 2021 and 2022; the exception was July, when the air temperature was 4.7°C greater than the long-term average. Thus, meteorological conditions during the autumn-winter and spring-summer growing seasons of the above years were generally satisfactory for biological processes in the soil and the development of winter wheat bioproductivity.

After the onset of the ontogenetic stages of spring tillering and wax ripeness (~75% of plants in one of these stages), soil samples were collected (depth 0-30 cm; row spacing – bulk soil) from experimental variants A-1–A-7, B-1–B-7, and prepared

for *in vitro* testing of the soil traits described in this study (DSTU ISO 11464-2001, 2003). The content of N-NO<sub>3</sub><sup>-</sup> was determined potentiometrically according to DSTU 4725:2007 (2008), N-NH<sub>4</sub><sup>+</sup> – using Nessler’s reagent (DSTU 4729:2007, 2008); the total content of the 2 listed forms of nitrogen in the soil was denoted as inorganic nitrogen N<sub>IN</sub> = N-NO<sub>3</sub><sup>-</sup> + N-NH<sub>4</sub><sup>+</sup>. The content of easily hydrolysed nitrogen (N<sub>EH</sub>, according to Kornfield) was measured according to DSTU 7863:2015 (2016), the content of carbon of labile humus (C<sub>LH</sub>, soluble in 0.2 M NaOH) was measured according to the Tyurin method – DSTU 4732:2007 (2008). N<sub>IN</sub>, N<sub>EH</sub>, C<sub>LH</sub> were calculated at mg kg<sup>-1</sup> of air-dried soil. To prepare the Nessler’s reagent, the study used the corresponding procedure presented in S. Abdelwahed *et al.* (2021), which the authors of the present study modified as follows: 45.50 g of HgI<sub>2</sub> “CHDA” (Chemlaborreactor LLC) was mixed with 35.00 g of KJ “CHDA” (Sphere Seven LLC), 50.00 ml of double-distilled water was added, and gradually stirred in 112.00 g of cold KOH “CHDA” (Sphere Seven LLC) + double-distilled water until the components were completely dissolved; stirring and cooling, the volume was gradually brought to 800.00 ml, and then to 1.00 L; left in the dark for ~72 h. The resulting reagent was filtered (ashless filter “Blue Ribbon”); therewith, the neck of the funnel contained glass wool, previously washed with dilute solutions of HCl, distilled water, and double-distilled water.

To determine the *in vitro* urease activity (urease, urea aminohydrolase, EC 3.5.1.5) in soil (ivUA) (0-30 cm; row spacing – bulk soil), the study employed typical approaches for testing this activity in soils, plant seeds, microorganisms, specifically, soil treatment with toluene (Cheng *et al.*, 2020; Tahir *et al.*, 2023), a reaction mixture based on 0.2-0.5 M phosphate-buffered saline (PBS) with pH ≈ 7.0, incubation temperature 30-38°C, incubation time 24 h (Zusfahair *et al.*, 2021; Magomya *et al.*, 2022), photometric measurement of NH<sub>4</sub><sup>+</sup> concentration with Nessler’s reagent at λ ≈ 400 nm (Nathan *et al.*, 2020). Briefly, ~5.00 g of air-dry soil, 10.00 ml of PBS (pH 6.7), 0.50 ml of toluene, 10.00 ml of 10.00% urea solution were added to an Erlenmeyer flask, sealed with cork stoppers, incubated at 30°C for 24 h; after incubation, 15.00 ml of 1 M KCl solution were added (reaction stop + NH<sub>4</sub><sup>+</sup> displacement); the contents of the tube were centrifuged at 3 × 10<sup>3</sup> rpm; an aliquot of the supernatant was significantly diluted with double-distilled water in analytical tubes, and a solution of K, Na-tartrate was added (the last 2 steps were performed in such a way as to leave room only for the Nessler reagent); 5–10 min after the addition of Nessler’s reagent, the extinction of the solution was measured at λ = 400 nm using an electrophotocolourimeter CFC-2. The value of ivUA was calculated in mg NH<sub>4</sub><sup>+</sup> kg<sup>-1</sup> of air-dried soil hour<sup>-1</sup>. The toluene, urea, K, Na-tartrate used in this method were of AR grade (Sphere Seven LLC), the phosphate components of PBS, KCl were of

chemically pure grade (Sphere Seven LLC); all solutions were prepared with double-distilled water.

To determine the *in situ* proteolytic activity (isPA) in the field using the method of Mishustin, Nikitin, and Vostrov (described by Khaziev) (Dubytska *et al.*, 2022), a gelatine-containing coating of medical X-ray film was used as a substrate. The film was placed vertically in the soil (up to 30 cm deep from the bottom edge; row spacing) and left for 4–8 days, pressed down from the sides (so that the middle of this time interval was approximately at the beginning of the onset of the ontogenesis phases of spring tillering and wax ripeness, i.e., ~75% of plants in one of these phases). After the film was excavated, it was placed in chilled plastic containers and transported to the laboratory. Before and after excavation, the film was weighed on a Radwag AS 220/R2 analytical balance (Poland) ( $\pm 0.0001$  g); before weighing the film with the already fermented gelatine-containing coating, the substrate carrier was lightly washed from the soil with a moderate stream of tap water and then air-dried for several hours. The isPA was calculated as the ratio of the film weight after and before application, expressed as %. All the above measurements were performed in 3 analytical (or “field” for isPA) parallels, and in 3 replicates ( $n = 6$ ). The obtained individual data for each indicator in the ontogenetic phases of spring tillering and wax ripeness were averaged among themselves for the spring and summer vegetation in 2021 or 2022. Thus, sets of averaged individual data ( $n = 6$ ) were obtained for the corresponding periods of plant development for years 1 and 2.

In grassroots approaches, such as genetic studies, at least 8–10 random or representative plants per plot or replication are sufficient to record ear productivity of agricultural cereal plants, specifically, grain weight per ear, *etc.* (Zhai *et al.*, 2020). In the present study, after the full grain ripeness stage (~75% of plants), 6 ears of grain were selected from productive shoots of winter wheat plants per replication, and in 3 replications ( $n = 18$ ) to estimate the grain weight per plant. Grain weight was measured on an analytical balance ( $\pm 0.0001$  g) (Radwag AS 220/R2, Poland). Grain mass per sown area (GM,  $\text{kg m}^{-2}$  of the sowing area) was calculated as the product of grain mass per plant and the number of productive shoots per  $\text{m}^2$ . The final values of each soil and plant trait for the 2 years of research were obtained by averaging the corresponding values (for  $n = 6$  or  $n = 18$ ; year 1, year 2) for the spring-summer growing season in 2021 and 2022.

The assessment of statistical validity  $p$  of pairwise differences between the numerical sizes of the analysed indicators in experimental variants A-1–A-7, B-1–B-7 was assessed using the Student’s *t*-test calculated as described by S. Brown *et al.* (2020); the appraisal of statistical soundness  $p$  of differences between the data in the groups of experimental variants A-1–A-7, B-1–B-7, and in the combined category A-1–B-7 were performed using ANOVA; the grades listed were executed in Microsoft Office Excel 14.0.7128.5000. The indicated

differences were considered significant if  $p < 0.05$ . The construction and analysis of the full form of the general linear model (GLM) and its not full form (without factor(s)) for the dependence of GM on the continuous predictors (covariates – random effects of variation  $N_{IN}$ ,  $N_{EH}$ ,  $C_{LH}$ , isPA, ivUA), categorical variable (factor – fixed effects of variation caused by experimental variants A-1–A-7, B-1–B-7) were performed on non-standardised individual (the continuous predictors) and neighbourhood averages (the categorical variable) in the experimental variation series of each experimental variant using Statistica Version 10 (StatSoft Inc). The corresponding data were presented only for the not full form of GLM. The Pearson product-moment correlation coefficient (PMC; based on the arithmetic means of each experimental variant), the corresponding  $p$  values, exploratory factor analysis (EFA) with dependent variable (GM) for 6 and 2 factors (based on the standardised means for each experimental variant; extraction – principal component analysis (PCA); rotation – varimax normalised) were calculated in the same software package. For the above methods of factor extraction and rotation, the study employed the options built into Statistica Version 10 (StatSoft Inc.) (specifically, Statistics → Multivariate exploratory techniques → Factor analysis → Variable selection → ... → Advanced → Extracting method: Principal component analysis → ... → Factor rotation: Varimax normalised). To obtain a variation series of 6 individual GM data per experimental variant, in the original variation series of 18 individual GM data per variant, each 3 neighbouring individual data were averaged.

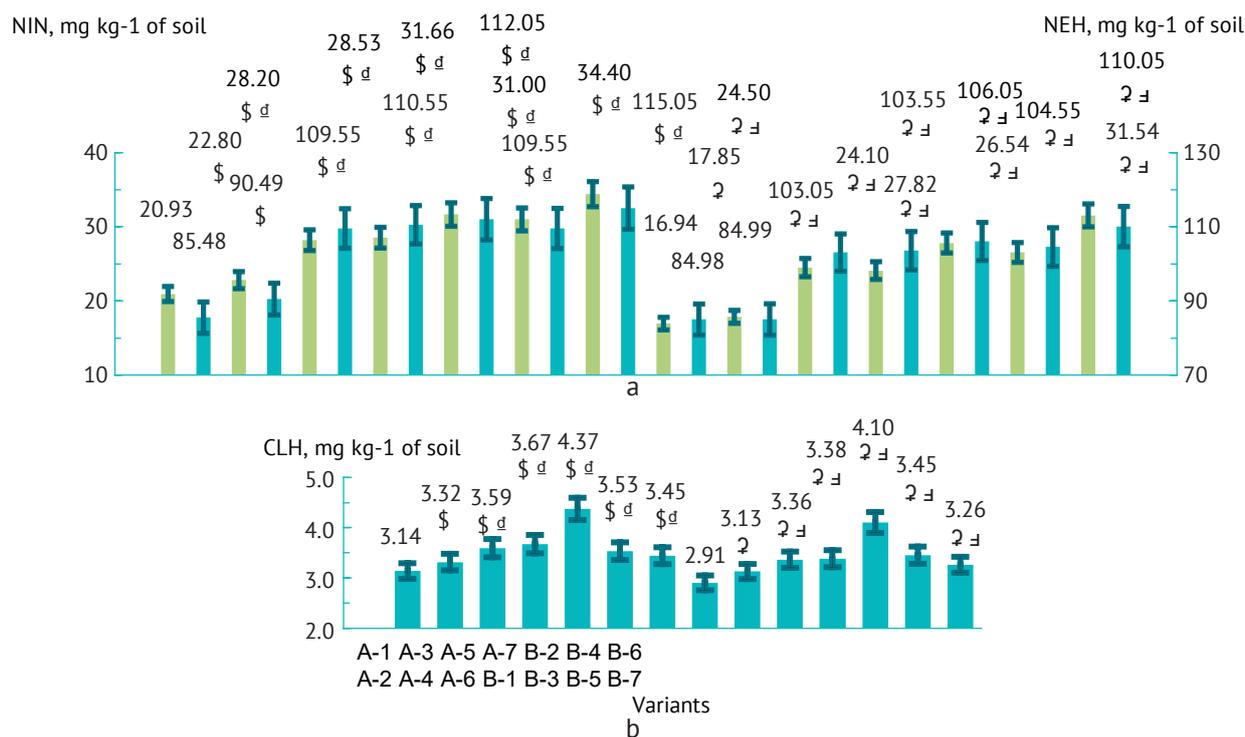
The results of the EFA (2 factors) served as the basis for a preliminary prediction of potential causal relationships between the variables under study. For a more detailed assessment of the hypothesised causal relationships between predictors and response functions, simultaneous equations models (Eqs.), designated as SIEMs (from non-standardised averages for each trial) in the GNU Regression, Econometrics and Time-series Library package (GRET 2021b; a free Unix-like operating system developed by the GNU Project); only recursive SIEMs among them were selected for the categories of resulting SIEMs. Since the 3-stage least squares (3SLS) approach to SIEMs was found to be heteroscedastic in some cases (the Breusch-Pagan test), the authors of the present study employed the weighted least squares (WLS) method (i.e., essentially, the weighted two-stage least squares W2SLS method) with iterative estimation. Additionally, in the case of 3SLS models, significant over-identification (the Hansen-Sargan test) was demonstrated several times. For the final SIEMs, the 2-stage least squares (2SLS) method provided mostly almost the same estimates as WLS, but the latter approach was still more reliable. Only the estimates built into GRET 2021b were used as typical estimates of the statistical effectiveness of SIEMs. The values of Fisher’s Criterion (FC) and their statistical validity for each Eq. in SIEMs were estimated in Microsoft

Office Excel 14.0.7128.5000 according to D.M. Levine *et al.* (2021). During the theoretical analysis, it became necessary to combine 2 Eqs. with semantic and mathematical mutual inverse causality within one system (e.g.,  $C_{LH} \rightarrow ivUA, ivUA \rightarrow C_{LH}$ ). The analysis of such SIEM using seemingly unrelated regressions (SUR), 2SLS, 3SLS, WLS approaches did not reveal any successful variance models. In this regard, two types of independent Eqs. were found ( $C_{LH} \rightarrow ivUA; ivUA \rightarrow C_{LH}$ ) using the Ordinary Least Squares (OLS) method (OLM) in GRETL 2021b. In addition, combining the last of the listed types of Eqs. into a single model within the assumed “subordination” using the 4 methods listed above did not lead to the formation of valid SIEMs. Therefore, the independent Eqs. derived by OLM were fully analysed (including FC) in GRETL 2021b and left for further interpretation. The study followed the standards of the Convention on Biological Diversity (1992) and the Convention on Trade in Endangered Species of Wild Fauna and Flora (1979).

### RESULTS

The study found that under the conditions of the environmentally safe technologies in the soil of variants

of groups A (A-2–A-7) and B (B-2–B-7), there was a significant ( $p < 0.001-0.05$ ) increase in the content of  $N_{IN}$  by 8.93–64.36%, 5.37–86.19%, relative to the control variants A-1, B-1, respectively (Fig. 1, a). Analogously, statistically significant ( $p < 0.001$ ) increases in  $N_{EH}$  content in the variants of groups A-2–A-7 and B-3–B-7, compared to the indicated controls, were 5.86–34.59%, 21.26–29.50%, respectively. In the case of B-2, the last of the listed indicators did not undergo statistically significant changes ( $p > 0.01$ ), compared to B-1. The values of both of the listed agrochemical features of soil N-state were statistically significantly ( $p < 0.001$ ) greater by 23.68–76.69% (for  $N_{IN}$ ), 21.06–29.49% (for  $N_{EH}$ ) under the conditions of the ecologically safe technologies A-3–A-7, B-3–B-7 vs. A-2, B-2, respectively (Fig. 1, a). Furthermore, in the variants A-2–A-7, B-2–B-7, there was an increase in the level of  $C_{LH}$  by 5.73–39.17%, 7.93–41.38% ( $p < 0.001-0.01$ ), relative to A-1, B-1, respectively (Fig. 1, b); in addition, the ecologically safe technologies A-3–A-7, B-3–B-7 caused an increase in the size of this indicator by 3.92–31.63%, 4.15–30.99% ( $p < 0.001-0.05$ ) compared to A-2, B-2, respectively.



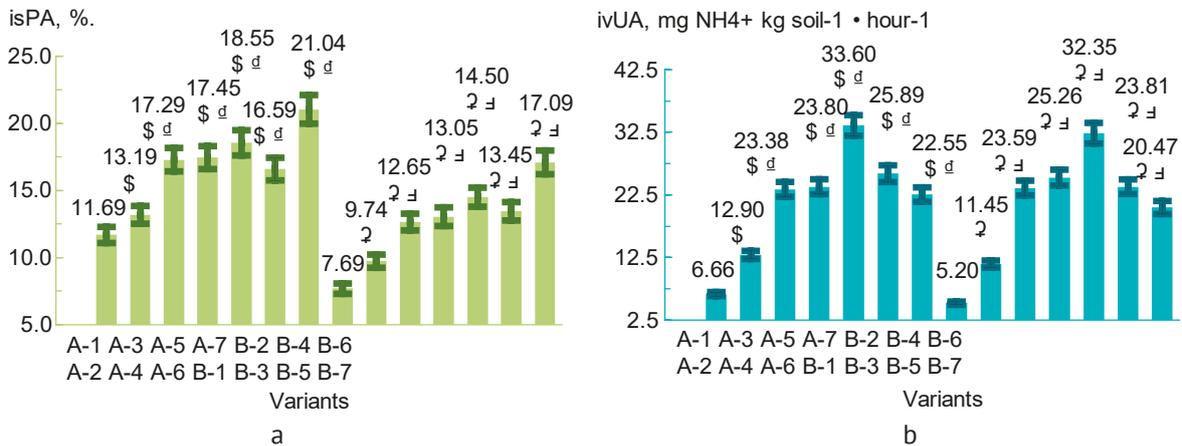
**Figure 1.** Variability of inorganic nitrogen ( $N_{IN}$ ), easily hydrolysable nitrogen ( $N_{EH}$ ) (a) and C of labile humus ( $C_{LH}$ ) (b) in the soil under winter wheat under the research variants

**Note:** A-1, B-1 – the controls of variant groups A, B with pea and fodder beans as precursors, respectively; A-2–A-7 or B-2–B-7 – the ecologically safe technologies of variant groups A or B, respectively; column height in the diagram = arithmetic mean (mean,  $M$ );  $n = 6$ ; data averaged by ontogenetic stages spring tillering, wax ripeness, and for 2021–2022), error bars =  $\pm$  standard error ( $SE, m$ ); numbers next to the levels of  $M \pm m$  – numerical values of the corresponding means; \$ or ? – the statistical validity of differences between the study variants A-2–A-7 and A-1 or B-2–B-7 and B-1 –  $p < 0.001-0.05$ ;  $\$$ ,  $\$$  – the statistical reliability of differences between the variants A-3–A-7 and A-2 or B-3–B-7 and B-2 –  $p < 0.001-0.05$ ; the statistical soundness of differences between the data in ANOVA for A-1–A-7, B-1–B-7 –  $p < 0.001-0.003$ , A-1–B-7 –  $p < 0.001$

**Source:** developed by the authors of this study

All the ecologically safe technologies used in variants A-2–A-7, B-2–B-7 caused a statistically significant ( $p < 0.001$ ) increase in isPA by 1.50–9.35%, 2.05–9.40% vs. A-1, B-1 controls, respectively (Fig. 2, a); at the same time, the increase of the indicated soil EA under conditions A-3–A-7, B-3–B-7 by 3.40–7.85%, 2.91–7.35% ( $p < 0.001$ ) was found compared to A-2, B-2,

respectively. Additionally, the ecologically safe factors in variants A-2–A-7, B-2–B-7 caused a statistically significant ( $p < 0.001$ ) increase in ivUA by 93.69–404.50%, 120.19–522.12%, relative to controls A-1, B-1, respectively (Fig. 2, b); ivUA increases in A-3–A-7, B-3–B-7 when compared with A-2, B-2, were 74.81–160.47%, 78.78–182.53% ( $p < 0.001$ ), respectively.



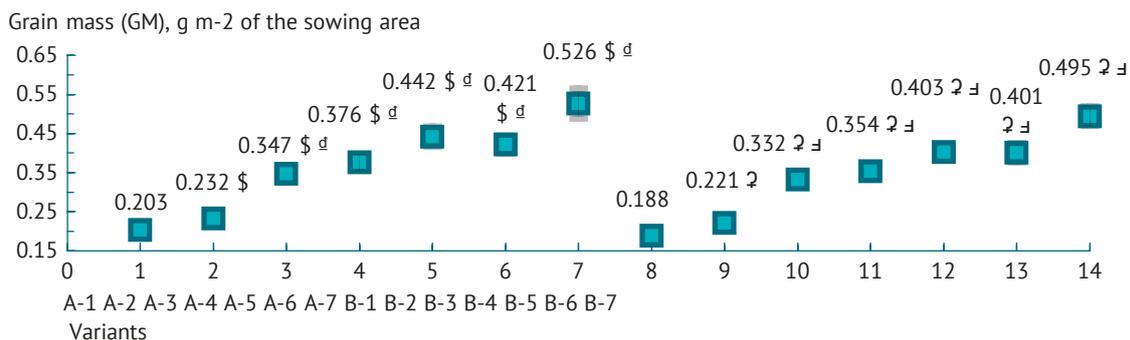
**Figure 2.** Changes in the arithmetic averages of in situ proteolytic activity (isPA) (a) and in vitro urease activity (ivUA) (b) in the soil under winter wheat depending on the research variant

**Note:** A-1, B-1 – the controls of variant groups A, B with pea and fodder beans as precursors, respectively; A-2–A-7 or B-2–B-7 – the ecologically safe technologies of variant groups A or B, respectively; column height in the diagram = arithmetic mean (mean,  $M$ ;  $n = 6$ ; data averaged by ontogenetic stages of spring tillering, wax ripeness, and for 2021–2022), error bars =  $\pm$  standard error (SE,  $m$ ); numbers next to the levels of  $M \pm m$  – numerical values of the corresponding means; \$ or ? – the statistical reliability of differences between the variants A-2–A-7 and A-1 or B-2–B-7 and B-1 –  $p < 0.001$ ; \$, ? – the statistical validity of differences between the variants A-3–A-7 and A-2 or B-3–B-7 and B-2 –  $p < 0.001$ ; the statistical soundness of differences between the data in ANOVA for A-1–A-7, B-1–B-7, A-1–B-7 –  $p < 0.001$

**Source:** developed by the authors of this study

Each of the ecologically safe technologies used in variants A-2–A-7, B-2–B-7 provided a statistically significant ( $p < 0.001$ ) increase in GM within 14.29–159.11%, 17.55–163.30%, compared to controls A-1, B-1, respec-

tively (Fig. 3); the ecologically safe factors in variants A-3–A-7, B-3–B-7 caused the levels of increment of this indicator by 49.57–126.72%, 50.23–123.98% ( $p < 0.001$ ), relative to A-2, B-2, respectively.



**Figure 3.** Grain mass (GM) per sowing area of winter wheat depending on the research variant

**Note:** A-1, B-1 – the controls of variant groups A, B with pea and fodder beans as precursors, respectively; A-2–A-7 or B-2–B-7 – the environmentally safe technologies of variant groups A or B, respectively; “shaded square” symbols – the arithmetic mean (mean,  $M$ ;  $n = 18$ ; ontogenetic phase of wax ripeness; averaged data for 2021–2022), error bars =  $\pm$  standard error (SE,  $m$ ); numbers next to the “shaded square” symbols – numerical values of the corresponding means; \$ or ? – the statistical validity of differences between the research options A-2–A-7 and A-1 or B-2–B-7 and B-1 –  $p < 0.001$ ; \$, ? – the statistical reliability of differences between the variants A-3–A-7 and A-2 or B-3–B-7 and B-2 –  $p < 0.001$ ; the statistical soundness of differences between the data in ANOVA for A-1–A-7, B-1–B-7, A-1–B-7 –  $p < 0.001$

**Source:** developed by the authors of this study

The one-factor (paired) linear correlation analysis showed the presence of statistically valid ( $p \leq 0.001-0.05$ ) medium and close interdependencies between GM and  $N_{IN}$ ,  $N_{EH}$ ,  $C_{LH}$ , isPA, ivUA with PMC values of 0.5500-0.9473 (Table 1). Therewith, the PMC values for the mutual subordinations between the last 5 variables listed were

also significant and statistically reliable (0.6055-0.9441;  $p \leq 0.001-0.05$ ). Such results most likely reflect multicollinearity within the correlation matrix under study. This substantially hinders the development of even the conceptual configuration of the causal relationships between GM and the N- and C-soil indicators presented here.

**Table 1.** Estimates of effect sizes, parameters, power, and efficiency for the not full form of GLM dependence of GM on the studied soil indices and the Pearson product-moment correlation coefficient (PMC) between these traits

| Index  | GLM ( $n = 6 \times 14$ ) |                       |                     |                        | Product-moment correlation coefficient (PMC) |                      |                     |                     |                     |
|--|---------------------------|-----------------------|---------------------|------------------------|--|----------------------|---------------------|---------------------|---------------------|
|  | Partial $\eta^2$          | Parameter             | $\beta$             | OP ( $\alpha = 0.05$ ) | GM   | $N_{IN}$             | $N_{EH}$            | $C_{LH}$            | isPA                |
| Intercept  | 0.5498 <sup>æ</sup>       | 582.202 <sup>æ</sup>  | –                   | 1.0000 <sup>æ</sup>    | –  | –                    | –                   | –                   | –                   |
| $N_{IN}$   | 0.1607 <sup>æ</sup>       | 24.273 <sup>æ</sup>   | 1.217 <sup>æ</sup>  | 0.9684 <sup>æ</sup>    | 0.9473 <sup>æ</sup>                          | –                    | –                   | –                   | –                   |
| $N_{EH}$   | 0.1285 <sup>æ</sup>       | -4.871 <sup>æ</sup>   | -0.638 <sup>æ</sup> | 0.9176 <sup>æ</sup>    | 0.9347 <sup>æ</sup>                          | 0.9441 <sup>æ</sup>  | –                   | –                   | –                   |
| $C_{LH}$   | 0.3232 <sup>æ</sup>       | -155.755 <sup>æ</sup> | -0.711 <sup>æ</sup> | 1.0000 <sup>æ</sup>    | 0.5500 <sup>ç</sup>                          | 0.6224 <sup>ç</sup>  | 0.6500 <sup>ç</sup> | –                   | –                   |
| isPA   | 0.0015                    | -2.358                | -0.078              | 0.0630                 | 0.8607 <sup>æ</sup>                          | 0.9608 <sup>æ</sup>  | 0.8965 <sup>æ</sup> | 0.6055 <sup>ç</sup> | –                   |
| ivUA   | 0.4019 <sup>æ</sup>       | 10.027 <sup>æ</sup>   | 0.743 <sup>æ</sup>  | 1.0000 <sup>æ</sup>    | 0.7733 <sup>æ</sup>                          | 0.7553 <sup>æ+</sup> | 0.8567 <sup>æ</sup> | 0.8669 <sup>æ</sup> | 0.6732 <sup>è</sup> |
| Separate performance indicators for the not full form of GLM |                           |                       |                     |                        |  |                      |                     |                     |                     |
| $R^2$  | 0.7564                    |                       |                     |                        |  |                      |                     |                     |                     |
| $R^2(\text{Adj})$  | 0.7408                    |                       |                     |                        |  |                      |                     |                     |                     |
| FC   | 48.4435                   |                       |                     |                        |  |                      |                     |                     |                     |
| $p$ (GLM)  | < 0.01                    |                       |                     |                        |  |                      |                     |                     |                     |

**Note:** GLM – General Linear Model (6 data per variant; 14 variants);  $\beta$  – standardised regression coefficient; OP – observed power;  $R^2$ ,  $R^2(\text{Adj})$ , FC,  $p$  (GLM) – the multiple coefficient of determination, adjusted multiple coefficient of determination, the Fisher coefficient, and the statistical reliability of the GLM; GM,  $N_{IN}$ ,  $N_{EH}$ ,  $C_{LH}$ , isPA, ivUA – the grain mass, the inorganic N, the easily hydrolysed N, the carbon of labile humus, in situ proteolytic activity, the in vitro urease activity; æ, æ+, è, ç – the statistical validity of  $p \leq 0.001$ ,  $p = 0.002$ ,  $p \leq 0.01$ ,  $p \leq 0.05$ , respectively

**Source:** developed by the authors of this study

The authors of this study suggested that for a better understanding of the systemic principles of the probable way GM is subordinated to  $N_{IN}$ ,  $N_{EH}$ ,  $C_{LH}$ , isPA, ivUA, it may be potentially expedient to generate the full form of the GLM (data not presented; the reasons for this are explained below) and/or the not full form of the GLM (without categorical variable); the results of the latter analysis are presented in Table 1. On the other hand, the relevance of this approach is confirmed by the properties of the structure of the used experimental data, namely: *i*) the above-mentioned multicollinearity within the correlation matrix of the data; *ii*) the deviation from the normal distribution of the  $N_{EH}$  parameter ( $p$  for  $H_1$  "there is a deviation from the normal distribution (ND)" in the case of the Doornik-Hansen, Lilliefors, and Shapiro-Wilk W-statistic tests was 0.0130-0.0222; in this situation, only the Jarque-Bera test revealed  $p = 0.4017$ ). Notably, logarithmisation of the data exacerbated the situation with the ND for  $\ln(N_{EH})$  and caused deviations from the ND for  $\ln(\text{ivUA})$  (approximately the same as in the case of the aforementioned mean variable).

In the resulting full form of the GLM ( $R^2 = 0.8351$ ,  $R^2(\text{Adj}) = 0.7894$ ,  $p < 0.01$ ; data not presented), only the values of the partial  $\eta^2$  for the intercept (= constant =  $b_0$ )

and the categorical variable (effects of variation due to the experimental variants) were statistically valid ( $p < 0.001-0.05$ ); the rest of the values of the partial  $\eta^2$  for the independent variables were statistically insignificant ( $p = 0.7162-0.9795$ ). In this form of GLM, the values of the parameters (regression coefficients  $b_0$ ,  $b_i$ ) for the intercept =  $b_0$  and the categorical variable for options A-1, A-2, A-7, B-1, B-2 were statistically valid ( $p < 0.001-0.05$ ). Therewith, the statistical significance of the variability of all the variables under study by variants was proved earlier using ANOVA (outside the full form of the GLM) and, on the other hand, the full form of the GLM was unable to characterise the subordination of GM to the predictors presented herein. The analysis of the not full form GLM showed significantly higher values of the partial  $\eta^2$ , parameter =  $b_0$  for the intercept, compared to the partial  $\eta^2$ ,  $b_i$  for the other regressors, and at the same time, statistical unreliability of the partial  $\eta^2$ ,  $b_4$  for isPA (Table 1). Clearly, this means that GM is more dependent on GM properties than on the regressors, and that GM is not caused by the variation of isPA. The synergistic effect of  $N_{IN}$ , ivUA, and the antagonistic effect of  $N_{EH}$ ,  $C_{LH}$  on GM development (by the signs of the parameter) are also evident

(Table 1). Therewith, certain inconsistencies between the not full form GLM and the correlation matrix of the data, as well as the lack of opportunities to predict the mediating effects of predictors, limit the potential of using this model to establish causality within the analysed system of variables.

The next step towards predicting causality was to apply EFA (with GM as the dependent variable) to find indicators that had the following set of properties: *i*) they shared variance with each other and simultaneously with GM; *ii*) their variance was separated from the unique variance and *iii*) from the error variance (Schreiber, 2021; Watkins, 2021). Furthermore, M.S. Sulaiman *et al.* (2021) demonstrated that regression of variables on the principal components (PCs) or the rotated PCs (essentially, on the factors in the FA) is characterised by a lower level of multicollinearity compared to regression of the original data; moreover,  $R^2$  is greater in the second case. Thus, EFA can reduce multicollinearity between variables. For factor extraction in this study, PCA was employed to reduce the number of items to a smaller number of representative uncorrelated components and to account for a larger proportion of the variance. Earlier, N. Shrestha (2021) confirmed that PCA solutions can lead to the emergence of items with cross-loadings; the variance of such an item can be explained by several factors. However, the authors of the current study considered it possible to

ignore this drawback to achieve the “essentially” desired purpose of EFA here. Based on the theoretical hypothesis that a simple latent structure should be at least partially based on uncorrelated measurements, the factor loadings matrix was orthogonally rotated using the varimax normalised method. An item was considered a statistically relevant factor identifier if it had a loading of  $\geq 0.7$  (Watkins, 2021).

The EFA results for the 6 factors (= the number of all variables) are presented in Table 2. The eigenvalues (EV) for the factors FA-1–FA-6 were 4.9895, 0.6947, 0.2206, 0.0673, 0.0148, 0.0130, and the corresponding values of the total variance (%) for the eigenvalues were 83.16, 11.58, 3.68, 1.12, 0.25, 0.22. For almost all variables within FA-1–FA-4, cross-loadings were found in the range of debatable values  $\sim 0.3$ – $\sim 0.6$  (Watkins, 2021), and no such loadings were found with values closer to significant (e.g., 0.60–0.69). As mentioned above, this may be conditioned by the PCA extraction of the factors, but it is also possible that it may be caused by the properties of the original data, specifically, multicollinearity between the analysed features (Kyriazos & Poga, 2023). However, statistically valid loadings occur only in the case of FA-1 and FA-2. Additionally, the EVs and total variance values for FA-1–FA-6 indicated the presence of a “breakpoint” (Schreiber, 2021) immediately after FA-1 or, at least, after FA-2, while the software used suggested leaving only FA-1 and FA-2.

**Table 2.** Loadings of the first six factors (the standardised variables; extraction: PCA; rotation: varimax normalised)

| Variable        | Factor loadings |         |        |         |         |         |
|-----------------|-----------------|---------|--------|---------|---------|---------|
|                 | FA-1            | FA-2    | FA-3   | FA-4    | FA-5    | FA-6    |
| GM              | 0.8062*         | 0.2590  | 0.4330 | 0.3088  | 0.0018  | 0.0106  |
| N <sub>IN</sub> | 0.8959*         | 0.3398  | 0.2526 | 0.0861  | 0.0152  | 0.1026  |
| N <sub>EH</sub> | 0.7749*         | 0.3739  | 0.4967 | -0.0054 | -0.1140 | -0.0043 |
| C <sub>LH</sub> | 0.2970          | 0.9453* | 0.1288 | 0.0326  | -0.0229 | 0.0012  |
| isPA            | 0.9301*         | 0.3371  | 0.1034 | -0.0664 | 0.0204  | -0.0756 |
| ivUA            | 0.4059          | 0.7116* | 0.5688 | 0.0494  | 0.0536  | 0.0114  |
| Expl.Var.       | 3.1710          | 1.8359  | 0.8489 | 0.1107  | 0.0170  | 0.0165  |
| Prp.Totl.       | 0.5285          | 0.3060  | 0.1415 | 0.0184  | 0.0028  | 0.0027  |

**Note:** GM – grain mass (dependent variable); N<sub>IN</sub>, N<sub>EH</sub>, C<sub>LH</sub>, isPA, ivUA – inorganic N, easily hydrolysed N, labile humus carbon, in situ proteolytic activity, in vitro urease activity (independent variables); FA-1–FA-6 – extracted factors; \* – loadings > 0.700000

**Source:** developed by the authors of this study

Elimination of FA-3 to FA-6 resulted in an increase in the amount of explained variance and the share of total variation for FA-1 and FA-2 (Table 3). The insignificant cross-loadings for FA-1 for the variables C<sub>LH</sub>, ivUA and for FA-2 for the variables GM and N<sub>IN</sub>, N<sub>EH</sub>, isPA were within 0.2833–0.5148. The authors of this study considered it expedient not to involve the last of these elements in the interpretation to preserve the possibility of forming and explaining simple, meaningful and mathematical constructs for the desired causal relationships between

the variables. Even though the ratio between the EVs for FA-1 and FA-2 ( $\sim 7.182 > 3$ ) (Schreiber, 2021) was found to be low (potentially, it may represent a relatively small part of the total variance, which, while not directly attributable to the model, can affect the observed estimates of such a construct in the experimental conditions and in reality). It is likely that in this case, the subordinations between the statistically reliable FA-2 items (C<sub>LH</sub>, ivUA) are at least partially independent of the key causal relationships between FA-1 items (GM, N<sub>IN</sub>, N<sub>EH</sub>, isPA).

**Table 3.** Loadings of the first two factors (extraction: PCA; rotation: varimax normalised), obtained based on the standardised variables

| Variable        | Factor loadings |         |
|-----------------|-----------------|---------|
|                 | FA-1            | FA-2    |
| GM              | 0.9065*         | 0.3370  |
| N <sub>IN</sub> | 0.9205*         | 0.3666  |
| N <sub>EH</sub> | 0.8604*         | 0.4661  |
| C <sub>LH</sub> | 0.2833          | 0.9364* |
| isPA            | 0.8975*         | 0.3243  |
| ivUA            | 0.5148          | 0.8228* |
| Expl.Var.       | 3.5600          | 2.1243  |
| Prp.Totl.       | 0.5933          | 0.3540  |

**Note:** GM (dependent variable) – grain mass; N<sub>IN</sub>, N<sub>EH</sub>, C<sub>LH</sub>, isPA, ivUA (independent variables) – inorganic N, easily hydrolysed N, labile humus carbon, in situ proteolytic activity, in vitro urease activity; FA-1, FA-2 – extracted factors; marked (\*) loadings > 0.700000

**Source:** developed by the authors of this study

Considering that the general organisation of the causal relationships between the variations of N-, C-soil indicators and GM studied herein under conditions of “central” mediation involving N<sub>IN</sub> (and/or N<sub>EH</sub>) or isPA (and/or ivUA), proposed in the INTRODUCTION section, is partly based on experimental data, partly on theoretical findings, and itself constitutes a certain theoretical generalisation, the following ways to elucidate the detailed structure of such subordination could be possible, namely: (i) exploratory structural equation modelling (ESEM) and its variants; (ii) confirmatory factor analysis structural equation modelling (CFA/SEM) and its variants; and (iii) path analysis models. However, the inclusion of latent constructs, complex types of variances, and combinations of mediator, moderator conditions between them, etc., in ESEM, CFA/SEM is more suitable for creating models on sufficiently large samples with a prominent level of generalisation of the final mathematical construction. Therewith, a series of simplifications in path analysis approaches, again, contribute too much to the “generalisation” of the resulting model. In the case of the more “local” patterns studied herein and the use of rarely used integrated environmentally safe technologies, it would be advisable to first characterise the causal relationships between variables with a somewhat greater level of specificity (as it were, narrowing down generalisations).

In this regard, the resulting factor structure (Table 3) was interpreted in the following 2 stages. Firstly, it is reasonable to assume that between the variables that significantly load (> 0.7) the factor 1, there may, overall, be causal subordination, where (i) N<sub>IN</sub> (or N<sub>EH</sub>) are predetermined and mediate the effects of each other and/or isPA on GM under the conditions of simultaneous action of N<sub>IN</sub>, N<sub>EH</sub>, isPA on GM, or (ii) not only N<sub>IN</sub> (or N<sub>EH</sub>) (mediating each other's effects), but also isPA (mediating the effects of both N<sub>IN</sub> and/or N<sub>EH</sub>) are predetermined, provided that N<sub>IN</sub>, N<sub>EH</sub>, isPA act simultaneously on GM. Secondly, variables that reliably load (> 0.7) the

factor 2 can be involved in semantic and mathematical reverse causality: variation in C<sub>LH</sub> causes the development of ivUA, and vice versa, variability in ivUA leads to the formation of C<sub>LH</sub>. Clearly, the above concept of causality between the above N- and C-soil condition traits and the trait of final biological productivity of winter wheat differs in a series of general points and nuances from the one presented in the INTRODUCTION section. A brief presentation of the finally proposed systems of causal subordination is as follows. Firstly: (i) N<sub>IN</sub>, N<sub>EH</sub>, isPA → GM; isPA, N<sub>EH</sub> → N<sub>IN</sub> (or isPA, N<sub>IN</sub> → N<sub>EH</sub>); isPA → N<sub>EH</sub> (or isPA → N<sub>IN</sub>), respectively; (ii) N<sub>IN</sub>, N<sub>EH</sub>, isPA → GM; N<sub>IN</sub>, N<sub>EH</sub> → isPA; N<sub>EH</sub> → N<sub>IN</sub> (or N<sub>IN</sub> → N<sub>EH</sub>), respectively. Secondly: C<sub>LH</sub> → ivUA (and/or ivUA → C<sub>LH</sub>).

All the above groups of causality were estimated by 3SLS, 2SLS, WLS, and, if necessary, SUR, OLS (the latter for the group of independent Eqs. (IEs) for the 2<sup>nd</sup> stage) using non-standardised data (specifically, to approximate concrete experimental conditions) to generate mathematically relevant, interpretable SIEMs or IEs. All SIEMs and IEs that characterise the causal and only mathematical relationships, respectively, between the studied soil and winter wheat traits listed in the previous paragraph are presented below. The SIEM-1 contains Eqs. (1), (2), (3):

$$\ln((0.5260 \times 50)/GM) = 15.0370^a - 1.4308^c \times \ln N_{IN} - 1.6750^c \times \ln N_{EH} + 0.6445^d \times \ln isPA, \quad (1)$$

$$\ln((34.4050/N_{IN}) \times 100) = 9.9197^a - 0.4669^a \times \ln isPA - 0.8193^b \times \ln N_{EH}, \quad (2)$$

$$\ln((115.0500/N_{EH}) \times 100) = 5.7005^a - 0.3667^a \times \ln isPA, \quad (3)$$

where <sup>a, b, c, d</sup> –  $p \leq 0.001, 0.01, 0.05, 0.1$ .

Here and in the following Eqs., the symbol “ln” means natural logarithm.

The SIEM-2 contains Eqs. (1) (see above), (4), (5) as follows:

$$\ln((115.0500/N_{EH}) \times 100) = 6.4714^a + 0.1204 \times \ln isPA - 0.6349^b \times \ln N_{IN}, \quad (4)$$

$$\ln((34.4050/N_{IN})) = 6.9292^a - 0.7673^a \times \ln isPA, \quad (5)$$

where  $^a, ^b - p \leq 0.001, 0.01$ .

The SIEM-3 contains Eqs. (1) (see above), (6), (7) as follows:

$$\ln((21.045/isPA) \times 100) = 7.4790^a - 1.4494^a \times \ln N_{IN} + 0.4825 \times \ln N_{EH}, \quad (6)$$

$$\ln((34.4050/N_{IN}) \times 100) = 13.3878^a - 1.8373^a \times \ln N_{EH}, \quad (7)$$

where  $^a$  is  $p \leq 0.001$ .

The SIEM-4 contains Eqs. (1) and (6) (see above), (8) as follows:

$$\ln((115.0500/N_{EH}) \times 100) = 6.3158^a - 0.4887^a \times \ln N_{IN}, \quad (8)$$

where  $^a$  is  $p \leq 0.001$ .

The group of independent Eqs. (IEs) (9), (10) is as follows:

$$\ln((33.5950/ivUA) \times 100) = 8.0052^a - 1.6055^a \times \ln(\ln^2(\ln^2(C_{LH}^2))), \quad (9)$$

$$\ln((4.3750/C_{LH}) \times 100) = 8.2472^a - 0.6816^c \times \ln ivUA + 0.0418^b \times \ln^4 ivUA - 0.0099^b \times \ln^5 ivUA, \quad (10)$$

where  $^a, ^b, ^c, ^d - p \leq 0.001, 0.01, 0.05, 0.1$ .

The symbols GM,  $N_{IN}$ ,  $N_{EH}$ , isPA, ivUA,  $C_{LH}$  denote the grain mass per sowing area, the content of inorganic N, easily hydrolysed N, the *in situ* proteolytic and *in vitro* urease activities, the labile humus C content, respectively. Eqs. (1)-(10) presented herein are variants of the linearised bounded exponential function denoted as log-ratio- $Y_{max}$ ; non-linearised versions of this function, on the one hand, asymptotically approach 0, and on the other hand, are limited to the maximum value of the dependent variable  $Y_{max}$  (the value in the numerator of the left-hand side of each Eq.). Depending on the grade  $b$ , this function can describe *i)* an exponential decline to 0; *ii)* an exponential increase, bounded from above by  $Y_{max}$  (in the case of an inverse relationship or a certain transformation of the variables). The bounded

exponential function can be found, specifically, in the publications of T.C. Han *et al.* (2021), T. Kolokolnikov and D. Iron (2021), M.M.T. Lakshani *et al.* (2023); a non-linearised form of the same Eq. was presented by M. Portnoi *et al.* (2021).

SIEM-1, SIEM-2 characterise the mediation of isPA effect on GM by  $N_{IN}$ ,  $N_{EH}$  traits in case of simultaneous action of these indicators of soil N status on the final biological productivity of winter wheat. SIEM-3, SIEM-4 reflect the mediator role of isPA in the additive effect of  $N_{IN}$ ,  $N_{EH}$ , isPA on GM, provided that the effects of  $N_{IN}$  and  $N_{EH}$  on the specified trait of the plant production process are mediated by each other, and all soil N-indicators considered here simultaneously affect GM. IEs (9), (10) characterise the independent mathematical determinations of log-ratio- $Y_{max}$  (ivUA) or log-ratio- $Y_{max}$  ( $C_{LH}$ ) by modified variables based on  $C_{LH}$  or ivUA, respectively.

Estimates of the statistical efficiency of SIEM-1-SIEM-4, IEs are presented in Table 4. For all Eqs. within SIEMs and IE (10), relatively low values of the regression standard error (RSE) were obtained (0.0332-0.0723), while for IE (9), the value of this characteristic was slightly greater (0.2745). The sizes of the coefficient of determination ( $R^2$ , the adjusted coefficient of determination ( $R^2_{(Adj)}$ ) for all Eqs. in SIEMs and IEs were significant and amounted to 0.7838-0.9670, 0.7658-0.9610, respectively, while the statistical validity of the Fisher coefficient (FC=40.2072-162.5170) was  $p < 0.001$ . The indicated ranges of the SIEMs and IEs estimates were accompanied by rather small values of the MAPE: 0.5612-2.8312 (Table 4). For all Eqs. within the SIEMs and IEs, the statistical reliabilities of the hypotheses  $H_1$  "heteroscedasticity occurs" and  $H_1$  "there is a deviation of the residuals from normality" were  $p > 0.005$ , reflecting the absence of heteroscedasticity and violations in the ND residuals. The determinants of the covariance matrices of the residuals for SIEM-1-SIEM-4 were quite close to 0; the corresponding ln-values were within -17.8911--16.1084, providing compelling evidence of linear dependencies between the individual Eqs. in these SIEMs. For the IE (10), the ln-determinant of the covariance matrix of the regression coefficients was -43.7045, while for the IE (9) it was -8.0508. This may suggest that IE (10) is much more consistent with a linear relationship between the predictors and the response variable than IE (9).

**Table 4.** Statistical performance estimates of the SIEMs and IEs

| No. of the SIEMs, IEs and Eqs. | RSE    | $R^2$  | $R^2_{(Adj)}$ | FC                     | MAPE   | Heteroscedasticity test (HST) | Residue normality test (RNT) | Ln(det) of the cov-matrix <sup>e</sup> |
|--------------------------------|--------|--------|---------------|------------------------|--------|-------------------------------|------------------------------|--|
| SIEM-1                         |        |        |               |                        |        |                               |                              |  |
| 1                              | 0.0723 | 0.9499 | 0.9348        | 63.1363 <sup>ae</sup>  | 1.4285 |                               |                              |  |
| 2                              | 0.0377 | 0.9670 | 0.9610        | 161.1814 <sup>ae</sup> | 0.6430 | 1.0000 <sup>d</sup>           | 0.2591 <sup>s</sup>          | -17.8911 <sup>dz</sup>                 |
| 3                              | 0.0479 | 0.7997 | 0.7830        | 47.9096 <sup>ae</sup>  | 0.7758 |                               |                              |  |
| SIEM-2                         |        |        |               |                        |        |                               |                              |  |

Table 4. Continued

| No. of the SIEMs, IEs and Eqs. | RSE     | R <sup>2</sup> | R <sup>2</sup> <sub>(Adj)</sub> | FC                     | MAPE    | Heteroscedasticity test (HST) | Residue normality test (RNT) | Ln(det) of the cov-matrix <sup>e</sup> |
|--------------------------------|---------|----------------|---------------------------------|------------------------|---------|-------------------------------|------------------------------|--|
| 1                              | -φ      | -φ             | -φ                              | -φ                     | 1.4285  |                               |                              |  |
| 4                              | 0.0332  | 0.9039         | 0.8864                          | 51.7170 <sup>ae</sup>  | 0.5612  | 1.0000 <sup>d</sup>           | 0.2888 <sup>s</sup>          | -17.8911 <sup>dz</sup>                 |
| 5                              | 0.0544  | 0.9312         | 0.9255                          | 162.5170 <sup>ae</sup> | 0.98755 |                               |                              |  |
| SIEM-3                         |         |                |                                 |                        |         |                               |                              |  |
| 1                              | -φ      | -φ             | -φ                              | -φ                     | 1.4285  |                               |                              |  |
| 6                              | 0.0664  | 0.9352         | 0.9235                          | 79.4192 <sup>ae</sup>  | 1.1983  | 1.0000 <sup>d</sup>           | 0.5302 <sup>s</sup>          | -16.1084 <sup>dz</sup>                 |
| 7                              | 0.0662  | 0.8980         | 0.8894                          | 105.5880 <sup>ae</sup> | 1.1869  |                               |                              |  |
| SIEM-4                         |         |                |                                 |                        |         |                               |                              |  |
| 1                              | -φ      | -φ             | -φ                              | -φ                     | 1.4285  |                               |                              |  |
| 6                              | -φφ     | -φφ            | -φφ                             | -φφ                    | 1.1983  | 1.0000 <sup>d</sup>           | 0.5081 <sup>s</sup>          | -17.4327 <sup>dz</sup>                 |
| 8                              | 0.03416 | 0.8980         | 0.8894                          | 105.5631 <sup>ae</sup> | 0.5742  |                               |                              |  |
| IE                             |         |                |                                 |                        |         |                               |                              |  |
| 9                              | 0.2745  | 0.7838         | 0.7658                          | 43.5126 <sup>ae</sup>  | 2.8312  | 0.4407–0.7132 <sup>†</sup>    | 0.7478 <sup>ds</sup>         | -8.0508 <sup>ds</sup>                  |
| 10                             | 0.0335  | 0.9234         | 0.9005                          | 40.2072 <sup>ae</sup>  | 0.32962 | 0.5068–0.8590 <sup>†</sup>    | 0.7504 <sup>ds</sup>         | -43.7045 <sup>ds</sup>                 |

**Note:** RSE, R<sup>2</sup>, R<sup>2</sup><sub>(Adj)</sub>, MAPE, FC – the regression standard error, the coefficient of determination, the adjusted coefficient of determination, the mean absolute percentage error, the Fisher coefficient, respectively; <sup>e</sup> – the natural logarithm of the determinant of the covariance matrix; <sup>ae</sup> – the statistical reliability of the FC –  $p < 0.001$ ; φ or φφ – the same values (sizes) of efficiency estimates, as in the case of the previous Eq. (1), SIEM-1 or Eq. (6), SIEM 3; <sup>d</sup>, <sup>s</sup> – the sizes (quantities) of the statistical validity  $p$  of the hypothesis  $H_1$  (HST (Breusch-Pagan)): “heteroscedasticity occurs”, and  $H_1$  (RNT (Doornik-Hansen)): “there is a deviation from normality”; <sup>†</sup>, <sup>‡</sup>, <sup>§</sup> – the intervals of sizes (quantities) of the statistical validity  $p$  of the hypothesis  $H_1$  (HST (White-only squares and the usual full versions, Breusch-Pagan regular and robust versions)): “heteroscedasticity occurs”, and  $H_1$  (RNT): “there is a deviation from normality”, respectively; <sup>e</sup> – the natural logarithm of the determinant of the covariance matrix of residuals of the SIEM or of the covariance matrix of the regression coefficients of each independent OLM-Eq; <sup>ds</sup>, <sup>dz</sup> – obtained from the covariance matrix of the residuals of different Eqs. of each SIEM or from the covariance matrix of the regression coefficients of each independent OLM-Eq, respectively

**Source:** developed by the authors of this study

Considering that for Eqs. (2), (3), (4), (5) within SIEM-1, SIEM-2, the RSE values (0.0332-0.0544), the MAPE range (0.5612-0.98755), and the values of the ln-determinant of the covariance matrix of residuals (-17.8911) are smaller compared to the corresponding estimates for Eqs. (6), (7), (8) in SIEM-3, SIEM-4 (0.03416-0.0664; 0.5742-1.1983; -17.4327--16.1084) (Table 4), it is reasonable to assume that SIEM-1, SIEM-2 may have a certain priority over SIEM-3, SIEM-4. However, the comparison of these Eqs. in the above groups of models by R<sup>2</sup>, R<sup>2</sup><sub>(Adj)</sub> (0.7997-0.9670, 0.7830-0.9610 vs. 0.8980–0.9352, 0.8894-0.9235, respectively) (Table 4) rather testifies in favour of the advantages of SIEM-2 over the rest of the analysed SIEMs. Still, it is possible that SIEM-1 will become relevant if and when Eqs. (2), (3) still dominate Eqs. (4), (5) under experimental and/or real field conditions. Nevertheless, SIEM-3, SIEM-4 can make a lot of sense in situations where there are various factors, stochastic disturbances that will cause deviations from the ND residuals of the considered SIEMs (greater  $p$  values (lower  $\chi^2$ ) for the  $H_1$  (residual normality test): “there is a deviation from normality” for SIEM-3, SIEM-4 are more difficult to reject than the corresponding smaller  $p$  values for SIEM-1, SIEM-2).

Significantly smaller (~ by an order of magnitude) RSE and MAPE, greater R<sup>2</sup>, R<sup>2</sup><sub>(Adj)</sub>, and substantially smaller values of the natural logarithm of the covariance matrix of the regression coefficients for IE (10), compared to IE (9) (Table 4) argue for the probable advantages of the log-ratio- $Y_{\max}$  ( $C_{LH}$ ) dependence on ivUA over the log-ratio- $Y_{\max}$  (ivUA) dependence on  $C_{LH}$ , however, without specifying causality. This assumption is also supported by a comparison of the values of the log likelihood, Akaike, Schwartz, and Hennane-Queen criteria (30.0500, -52.3366 to -49.5438 for IE (10) versus -0.686762, 5.2552 to 6.6516 for IE (9), respectively). Therewith, for each of the regressors in IE (10), structural shifts were found by the Chow test (centre of the sample); the latter may reflect a partially imperfect model specification and, possibly, the effects of multicollinearity on the properties of this model.

Notably, the 3SLS method is typically more consistent and provides more asymptotic estimates for SIEM and SEM compared to 2SLS, OLS. This is not least conditioned by the fact that 3SLS is a combination of 2SLS and SUR (Tetteh, 2024). In this regard, 3SLS jointly estimates the entire model in a structural form and factors in the simultaneous cross-correlations between errors

(Butkus *et al.*, 2024). Such modelling properties allow adjusting the weighting matrix for potential heteroscedasticity problems in 3SLS regression equations using the covariance matrix of the residuals from 2SLS; the generalised least squares method is usually used for parameter estimation (Huda & Istiana, 2024). However, 2SLS is effective when there is a secondary predictor that is correlated with the problem predictor (even hypothetically) but not with the error term. In the case when stochastic disturbances in different equations are uncorrelated or there is no heteroscedasticity, the results of 2SLS are typically equivalent to 3SLS estimates (Tetteh, 2024). Notably, the advantages of 2SLS over 3SLS may substantially depend on the level of multicollinearity between the variables (Oduntan, 2023). It would not be unreasonable to assume that the priority of 2SLS, or even OLS vs. 3SLS, at least in part, may also be conditioned by contamination of variables by errors, specifically, with small samples, depending on the accuracy of identification of Eqs., the type of distribution, and sample size. Overall, it is possible that the combination of similar multicollinearity and identification problems, in addition to small sample sizes, were significant reasons for the preference of 2SLS (SIEM-1–SIEM-4) and OLS (IEs (9), (10)) over 3SLS.

Therewith, the approach to formulating and evaluating SEM, SIEM (even if it is 2SLS) can be considered as a task for an artificial neural network (ANN), and therefore it makes sense to consider SEM, SIEM as special cases of ANN (Pérez-Sánchez *et al.*, 2024). The latter clearly suggests the relevance of using SEM, SIEM to solve various problems, at least not of a large size, based on mathematical and statistical regularities and assumptions about causality between variables. It is natural to assume that the mentioned significance of SEM, SIEM is inherent in biological and agricultural scientific problems.

## DISCUSSION

The authors of the present study assumed that changes in  $N_{IN}$ ,  $N_{EH}$ ,  $C_{LH}$  in the soil of variants A-1–B-7 are the outcome of a complex interaction of physicochemical and biological processes initiated by plant residues (PR–FPS/FBS), mineral fertilisers (RMF/HMF) and/or bioeffectors (BS/HF/CF). Considering the following arguments, it is worth noting that the C:N ratio in biomass or straw (STR) of legumes is several times lower than in non-legumes (Watthier *et al.*, 2020; Grimm *et al.*, 2024) and may approach the threshold of 25:1 for this criterion in the substrate (S) (Cao *et al.*, 2021). Thus, it is likely that the addition of legume STRs alone (variants A-2, B-2) stimulated N-releasing microbial activity (MA), N-cycling, and gross mineralisation (Robertson & Groffman, 2024), providing energy for heterotrophic PR-degrading microflora (analogous to priming – PRM (Jesmin *et al.*, 2021)), although efficiency may be reduced under N deficiency due to N sequestration in the microbial biomass (MB) (Henneron *et al.*, 2020).

Reducing C:N in OM or adding N (also possibly  $N_{IN}$ ) can enhance net N mineralisation and weaken N immobilisation (Sawicka *et al.*, 2020; Cao *et al.*, 2021; Robertson & Groffman, 2024), which is known as PRM (Meng *et al.*, 2020) and was observed in A-3, B-3 (FPS/FBS + RMF). Such intensive treatments can increase PR and  $CO_2$ -production, but do not promote C-sequestration (Jesmin *et al.*, 2021). Mineralisation of OC (especially under N-stimulation) releases C,N-S to MB, enhancing MA, which decomposes PR, increasing  $N-NH_4^+$  and  $N-NO_3^-$  (observed for  $N_{IN}$  in A-3, B-3). The formation of  $N_{IN}$  is preceded by the formation of available SON from its turnover in soil and PR (Yan *et al.*, 2020). The evident partial overlap of the fraction of dissolved organic nitrogen (DON) with  $N_{EH}$  explains the unidirectional changes in  $N_{IN}$  and  $N_{EH}$  in the soil of the studied variants (Farzadfar *et al.*, 2021). Comparable changes in  $C_{LH}$  in A-3, B-3 are probably conditioned by the decomposition of SOM and SON, especially with PR.

The increase in the *in situ* PA (isPA) in A-2 and B-2, compared to A-1, B-1, was consistent with stimulated mineralisation due to a decrease in C:N in S and accumulation of  $N_{IN}$ ,  $N_{EH}$ ,  $C_{LH}$  (Jesmin *et al.*, 2021). Removal of N-limiting (N-LIM) can increase microbial attack on fresh OM (Henneron *et al.*, 2020). The levelling of C-LIM by available N (Luo *et al.*, 2021) explains the analogous changes in isPA,  $N_{IN}$ ,  $N_{EH}$ ,  $C_{LH}$  in A-3, B-3, relative to A-1, B-1, which is also consistent with the dependence of protein mineralisation on S and proteases (Greenfield *et al.*, 2020) and the correlation of PA with DON (Farzadfar *et al.*, 2021), and thus probably with  $N_{EH}$ . Unidirectional changes of the *in vitro* UA (ivUA) and isPA (at least in A-1–B-3) are predicted, specifically, due to the participation of UA in N cycling and based on the findings of J. Yang *et al.* (2020), M.I. Khan *et al.* Experiments by P. Tian *et al.* (2020) revealed the effect of STR ploughing depth on ivUA. The application of N fertiliser + rice STR increased ivUA (Sharma *et al.*, 2021), while commercial mulch decreased ivUA in long-term experiments (Sun *et al.*, 2021).

Changes in soil physicochemical properties in A-4–B-7 are potentially caused by a combination of PRM (N-stimulated mineralisation of crop residues, as in A-3, B-3) and rhizosphere PRM (rPRM), considering the significance of the relationship between the rhizosphere and bulk soil (Sun *et al.*, 2021). In A-4–A-6/B-4–B-6, rPRM could be initiated with BS, BS+HF or CF. BSs contain phytohormones and C,N-auxiliary compounds that stimulate metabolism, growth, and nutrient uptake (Franzoni *et al.*, 2022), and CFs may act similarly to BSs and HFs (Musych *et al.*, 2024). It was assumed that BS, BS+HF, CF stimulated N uptake, photosynthesis, wheat growth, and C-rhizodeposits release, which could enhance N cycling through rPRM degradation of SOM (Henneron *et al.*, 2020). This could have been combined with bulk soil N-PRM (A-3, B-3), causing an increase in  $N_{IN}$ ,  $N_{EH}$ ,  $C_{LH}$ , NME. However, the absence of substantial

differences in  $N_{IN}$ ,  $N_{EH}$ ,  $C_{LH}$ , isPA, ivUA in A-4, A-6, B-4, B-6 vs. A-3, B-3 reflects insignificant synergistic effects between the 2 types of PRM and, probably, the predominance of the influence of N-stimulated PR mineralisation. The power and duration of these technologies are clearly insufficient for C and N sequestration.

Conversely, A-5, B-5 had maximal  $C_{LH}$ , insignificant changes in  $N_{IN}$ ,  $N_{EH}$ , isPA, and a significant increase in ivUA (the latter complicates the assessment of N sequestration) compared to A-3, B-3. It was assumed that the synergistic effect of BS+HF in A-5, B-5 stimulated the rhizosphere turnover of SOM, probably activating C-cycles throughout the soil. Q. Ma *et al.* (2020) argued that N mineralisation and nitrification increase with decreasing N- and increasing C-LIMs. Therefore, the decrease in C-LIMs due to increased SOM turnover led to the accumulation of  $C_{LH}$  in A-5, B-5, while the intensity of N-turnover and  $N_{IN}$ ,  $N_{EH}$ , isPA were comparable to A-3, A-4, A-6 and B-3, B-4, B-6. Based on the hypothetical pattern of C sequestration under chronic N deposition (Lu *et al.*, 2021) and the findings of Q. Ma *et al.* (2020), partial enhancement of C- and weakening of N-LIMs in A-7, B-7 (HMF-apl.) was assumed. The rPRM from HF may not generally compensate for C-LIM in these variants, which was accompanied by a moderate decrease in  $C_{LH}$  turnover, but also a moderate increase in N mineralisation ( $N_{IN}$ ,  $N_{EH}$ , isPA); ivUA co-changed with  $C_{LH}$ .

Changes in N, C, and NME forms reflect the functioning and interaction of microbes and plants at the soil level, where intermediates (IM) and final products (FP) of these cycles are a source of plastic material and energy. MA, particularly NME, can influence crop productivity, which is consistent with the observed correlation (PMC) ( $N_{IN}$ ,  $N_{EH}$ ,  $C_{LH}$ ) – GM, as well as (isPA, ivUA) – GM. The significance of soil NME for plant productivity was confirmed by studies (Zhou *et al.*, 2020; Yang *et al.*, 2022; Garnaik *et al.*, 2022), which showed  $r > 0$  for (ivPA/UA) – (wheat/rice yield). The absence of GM dependence on isPA within the GLM used may be partially consistent with the findings of other studies (Nath *et al.*, 2021), where no significant single-factor dependence (rice yield) – ivPA was found. However, the authors of the present study believe that the results of the incompletely parameterised GLM provide insufficient information to understand the causal relationships between the considered regressors and GM.

The EFA results reflect that GM is dependent on  $N_{IN}$ ,  $N_{EH}$ , and isPA (factor 1, FA-1), while  $C_{LH}$  and ivUA form a separate FA-2, potentially affecting each other but not directly GM, which may reflect the partial decoupling of the N and C cycles and the asynchrony of N cycle elements (Ma *et al.*, 2020). The N-LIM of microorganisms may undergo a deepening under excessive available C (PR), which will stimulate compensatory N-mineralisation (Tan *et al.*, 2021; Qiu *et al.*, 2021; Cui *et al.*, 2022), explaining the spatial and temporal divergence of the N and C cycles. Admittedly, with the depletion of

labile fractions of plant SOM/SOM, microbes attack N-enriched SOM, activating N-mineralisation, which increases N availability. Conversely, the presence of C in low-energy compounds favours C-LIM. Thus, changes in the energy supply of microorganisms, affecting the C/N balance, limit N mineralisation and break the causality between microbial C and N availability/demand (Zhang *et al.*, 2021).

The link between  $C_{LH}$  and ivUA may be mediated by intensification of ureolysis, which increases N availability, promoting the development of microbial communities less capable of degrading recalcitrant C, leading to C sequestration and reduced NME activity. Indirect C-input from plant residues can stimulate EA, particularly UA (Tan *et al.*, 2021). Other studies have not found a direct correlation between rice/maize yields and ivUA (Nath *et al.*, 2021; Kalala *et al.*, 2022). The distribution of variables by FA-1, FA-2 partially revises the hypotheses about the causal relationships between soil characteristics and wheat GM. In method (i) (abbreviated: NME → agrochemical/physical-chemical soil characteristics → GM), the simultaneous additive effect of  $N_{IN}$ ,  $N_{EH}$ , isPA on GM is assumed (Eq. (1)), where each of the pools of  $N_{IN}$ ,  $N_{EH}$  depends on isPA and another pool (Eqs. (2), (3) in SIEM-1, Eqs. (4), (5) in SIEM-2). Thus, the effect of isPA on GM can be mediated through  $N_{IN}$  (depending on the ratio of isPA and  $N_{EH}$ ) or through  $N_{EH}$  (depending on the ratio of isPA and  $N_{IN}$ ).

To argue for a positive feedback loop (PFL) (method (ii); abbreviated: agrochemical/physical-chemical soil characteristics → NME activity → GM), the resource allocation theory is applied, according to which nutrients-LIM for microorganisms stimulates the production of eco-EA (EEA) for their assimilation (Luo *et al.*, 2021; Mori *et al.*, 2021), i.e.,  $EEA \propto 1/(\text{nutrient availability})$  (negative feedback loop (NFL), possible under C-LIM; L. Luo *et al.* (2021)). C-enrichment can limit microorganisms, causing an increase in EA for other nutrients, particularly N (Tan *et al.*, 2021), which could account for the PFL between EA and N and C forms found by P. Tian *et al.* (2020). Enhanced N mineralisation can stimulate NME more strongly than EA to produce C (Qiu *et al.*, 2021). The size of the hydrolase pool ( $V_{max}$ )  $\propto$  of the S concentration (Tan *et al.*, 2021). Causality method (ii) assumes a combination of simultaneous determination of GM by  $N_{IN}$ ,  $N_{EH}$ , isPA with the predetermination of isPA by the ratio  $N_{EH}/N_{IN}$  and mutual determination of  $N_{IN}$  and  $N_{EH}$ . Hence, Eq. (1) is combined with the mediation of the effect of the  $N_{IN}/N_{EH}$  balance through isPA on GM and the mediation of the effect of  $N_{EH}$  on GM through  $N_{IN}$  (or vice versa) (SIEM-3/SIEM-4). In causality mode (ii), GM simultaneously depends on  $N_{IN}$ ,  $N_{EH}$ , and isPA (Eq. (1)), while isPA is mediated by  $N_{EH}/N_{IN}$  (Eq. (6)), while  $N_{IN}$  and  $N_{EH}$  are mutually determined (Eqs. (7), or (8); SIEM-3/SIEM-4).

Eq. (1) showed the direct effect of  $N_{IN}$  and  $N_{EH}$  on GM growth and the inverse dependence of GM on isPA.

In SIEM-1, an increase in isPA and  $N_{EH}$  mediated an increase in  $N_{IN}$ , while an increase in isPA alone mediated an increase in  $N_{EH}$ . In SIEM-2, a decrease in isPA and an increase in  $N_{IN}$  could mediate the effect of  $N_{EH}$ , while an increase in isPA alone could cause an increase in  $N_{IN}$ . In SIEM-3/SIEM-4, an increase in  $N_{IN}$  and a decrease in  $N_{EH}$  were influenced by an increase in isPA, while an increase in  $N_{EH}$  caused an increase in  $N_{IN}$  (and vice versa). All these dependencies are statistically significant. The statistical significance of the relationship between  $C_{LH}$  and ivUA (IE 10) reflects correlation rather than causation, and  $C_{LH}$  may be influenced by other factors. The expected positive effect of  $N_{IN}$  and  $N_{EH}$  on GM (Eq. (1)) contrasts with the unexplained inverse relationship between isPA and GM. This contradiction is partially explained by the dependence of  $N_{IN}$  growth on isPA and  $N_{EH}$ , and  $N_{EH}$  growth on isPA (Eqs. (2), (3)), as well as the dependence of  $N_{EH}$  growth on isPA decline and  $N_{IN}$  growth, and  $N_{IN}$  growth on isPA increase (Eqs. (4), (5)).

The positive effect of  $N_{EH}$  and isPA on  $N_{IN}$  (Eq. (2)) suggests that protease-mediated mineralisation of S from  $N_{EH}$  (isPA) increases the available forms of  $N_{IN}$ , which is consistent with the concept of N-mineralisation (Robertson & Groffman, 2024) and the significance of SON/DON (Ma *et al.*, 2020; Yan *et al.*, 2020). This is also consistent with the role of soil TN (and likely SON) and MBN in controlling nitrification (Li *et al.*, 2020). The ability of isPA to stimulate  $N_{IN}$  accumulation (Eq. (2)) supports the concept that protein mineralisation rates depend on available S and protease production (Greenfield *et al.*, 2020), with the amount of S ( $N_{EH}$ ) being more significant. Interpreting  $N_{EH}$  as proteolysis products, its level increases with increasing isPA (Eq. (3)), which is also consistent with N-mineralisation (depolymerisation of SON by isPA leads to the accumulation of  $N_{EH}$ /DON; X. Sun *et al.*, 2021) and the effect of ivPA and other factors on SON (Yang *et al.*, 2020). The triggering effect of isPA on  $N_{EH}$  (Eq. (3)) and the consolidated effect of isPA and  $N_{EH}$  on  $N_{IN}$  (Eq. (2)) are manifested in Eq. (1) as additive stimulating effects of  $N_{IN}$  and  $N_{EH}$  on wheat GM with simultaneous suppressive effect of isPA on GM. It is unclear whether this is a direct effect of isPA on GM or a latent NFL between this EA and  $N_{IN}/N_{EH}$ , which is consistent with the theory of resource allocation in the soil.

In SIEM-2, the effect of isPA on  $N_{IN}$  and  $N_{EH}$  with the stimulating effect of the latter on GM and the NFL from isPA on GM are complex. The key is the activating effect of isPA on  $N_{IN}$  (Eq. (5)), simultaneously with the suppressive effect of isPA and the stimulating effect of  $N_{IN}$  on  $N_{EH}$  (Eq. (4)). The ability of isPA to increase  $N_{IN}$  (Eq. (5)) is consistent with the characteristics of N-mineralisation and proteolysis. The stimulatory effect of  $N_{IN}$  on  $N_{EH}$  (Eq. (4)) may be conditioned by PFL, which is consistent with the significance of SON turnover (Yan *et al.*, 2020) and soil N priority (Anas *et al.*, 2020), and is also consistent with FL control by  $N_{IN}$  of the BAN pool (Daly *et al.*, 2021). Enhanced microbial C supply and activation of

N-mineralisation could contribute to the development of PFL control of NME/isPA by  $N_{IN}$ . The stimulatory effect of  $N_{IN}$  on  $N_{EH}$  (as a product of isPA) may reflect the stimulation of isPA levels by  $N_{IN}$ , which is partially consistent with the findings of L. Greenfield *et al.* (2020). The inverse effect of isPA on  $N_{EH}$  may reflect a decrease in S with an increase in EA. Therefore, in SIEM-2, isPA stimulates  $N_{IN}$  (Eq. (5)), which increases  $N_{EH}$  (Eq. (4)), and the growth of both forms of N positively influences GM (Eq. (1)). The simultaneous increase of isPA balances the activating effect of  $N_{IN}$  on  $N_{EH}$  (Eq. (4)) and counteracts the favourable effects of these forms of N on GM (Eq. (1)).

For SIEM-3 and SIEM-4, the feedback between isPA and its products is significant. The stimulatory effect of  $N_{IN}$  on  $N_{EH}$  (Eq. (8), SIEM-4, as in Eq. (4), SIEM-2) may reflect the enhancement of isPA by FP proteolysis and N-mineralisation, which is consistent with the activating effect of  $N_{IN}$  on isPA (Eq. (6), SIEM-3/4). The direct relationship between  $N_{IN}$  and  $N_{EH}$  (Eq. (7), SIEM-3) reflects that  $N_{IN}$  increases with increasing  $N_{EH}$ . The inhibitory effect of  $N_{EH}$  (as products of isPA) on isPA (Eq. (6), SIEM-3/4) indicates FL. Therefore, in SIEM-3/4, an increase in  $N_{EH}$  (the result of isPA) causes an increase in  $N_{IN}$  (Eq. (7)), which can enhance isPA (Eq. (6)) and expand  $N_{EH}$  (Eq. (8)), while an increase in  $N_{EH}$  suppresses isPA. The indirect ratio of the effects of  $N_{IN}$  and  $N_{EH}$  on isPA increase can cause GM falloff, while the consolidated gain of these N forms is GM gain (Eq. (1)). The stimulatory effect of  $N_{IN}$  on  $N_{EH}$  can lead to an indirect decrease in GM gain by isPA; on the other hand, the expansion of  $N_{IN}$  can cause an increase in isPA, which will lead to GM falloff.

This feedback is different from the one proposed by X. Tan *et al.* (2021). However, the stimulatory effect of  $N_{IN}$  on  $N_{EH}$  and isPA (Eqs. (4), (8), (6)) is consistent with the ability of  $N_{IN}$  to increase ivPA, which is consistent with  $r > 0$  for  $N-NH_4^+ - ivPA$  (Sawicka *et al.*, 2020), but partially contradicts the  $r < 0$  found by them for  $N-NO_3^- - ivPA$ . These interpretations may be opposed by the effects of ivPA inhibition by high concentrations of  $NH_4^+$  or  $NO_3^-$  (Jesmin *et al.*, 2021) and a moderate  $r < 0$  between the rate of slow phase protein mineralisation and  $NO_3^-$  (Greenfield *et al.*, 2020), although a possible  $r > 0$  between N-cycle enzyme (NAG) activity and  $NO_3^-$  (Błońska *et al.*, 2021). The attenuation of isPA by  $N_{EH}$  is partially consistent with the resource allocation theory and the concept of decreasing NME activity with increasing available N forms (He *et al.*, 2021), where the size of the  $N_{EH}$  pool may reflect the availability of N. Additionally, E. Błońska *et al.* (2021) argued that mineralisation dominates immobilisation in the decomposition of N-rich detritus, which may lead to a decrease in NME production and  $r < 0$  between this EA and SON/DON/ $N_{EH}$  components, but  $r > 0$  between NAG and N light fractions is possible (Błońska *et al.*, 2020).

The statistical efficiency of IE (10) versus IE (9) and the greater nonlinearity of the latter reflect a probable dependence of  $C_{LH}$  on ivUA, rather than vice versa.

However, the activating effect of humic acids (HA) and vermicompost on ivUA is known to be accompanied by an increase in microbial population and C:N, while HA from weathered coal/leonardite can inhibit ivUA (Ampong *et al.*, 2022). IvUA is nonlinearly dependent on the mass fraction of soil macroaggregates (Zhao *et al.*, 2020), but the effect of initial OM and MBC on ivUA, as well as the dependence of SON on the latter, was not confirmed (Yang *et al.*, 2020). At the same time, an inverse relationship between ivUA and MBC was found (Sun *et al.*, 2021). There is evidence of the effect of ivUA on SOC components, including a significant effect on fine POC (Liu *et al.*, 2021) and participation in the pre-mortemisation of other SOC fractions. IvUA can mediate the effect of organic fertiliser on MBC and its effect on LOC, as well as the effect of organic fertiliser on LOC/UOC and their effect on SOC (Zhang *et al.*, 2022).

The obtained results of EFA, SIEMs, IEs do not show a clear direction of changes in ivUA and N conversion/mineralisation, unlike the study of organic tree mulching (Sun *et al.*, 2021). However, the PMC between ivUA –  $N_{IN}$  is partially consistent with the directly proportional ivUA –  $NH_4^+$  relationships (Sun *et al.*, 2021; Sawicka *et al.*, 2020), the high probability of ivUA –  $N_{IN}/NH_4^+/NO_3^-$  relationships (Sharma *et al.*, 2021), and the positive effect of  $NO_3^-$  on ivUA (Tian *et al.*, 2020). Inconsistencies between correlation and multivariate analyses, as well as ambiguities in their agreement with the literature, may be in favour of the expected spatial and temporal divergence of N and C cycle trajectories in all tested variants, which may be conditioned by *i*) the specific features of N- and C-mineralisation, PRM and the role of plants in each variant, *ii*) insufficient number of explanatory variables to reflect the relationships between N- and C-cycles in soil in relation to plant productivity. The application of PR with/without fertilisers and bioeffectors determined the direction of N- and C-mineralisation, where variations in  $N_{IN}$ ,  $N_{EH}$ , isPA were the probable causes of GM changes. All the analysed causal relationships were characterised by a positive effect of  $N_{IN}$  and  $N_{EH}$  on GM and a possible suppressive effect of isPA on GM. The increase in GM due to an increase in  $N_{IN}/N_{EH}$  can potentially be accompanied by a decrease in isPA (a possible latent NFL), which is partially consistent with the theory of resource allocation and the stoichiometry of N-mineralisation.

Considering the statistical efficiency of SIEMs, mediation of isPA effect on GM via  $N_{IN}/N_{EH}$  (SIEM-1/2) is more probable than mediation of N-forms effect on GM via isPA (SIEM-3/4). The most plausible is the stimulating effect of  $N_{IN}$  on isPA and the dependence of  $N_{EH}$  on the balance between isPA and  $N_{IN}$  (SIEM-2). Less relevant are the dependence of  $N_{EH}$  on isPA and the conditionality of  $N_{IN}$  on the consolidated effect of isPA,  $N_{EH}$  (SIEM-1). Statistically, the least reliable isPA control of the  $N_{EH}/N_{IN}$  ratio (SIEM-3/4) may be consistent with latent NFL and may be updated under certain conditions.

Simultaneously with the influence of N-characteristics on GM, there may be a separate dependence of  $C_{LH}$  on ivUA and other unidentified factors (IE 10), while the dependence of ivUA on  $C_{LH}$  (IE 9) is less likely. The reasons for the separation of  $C_{LH}$  and ivUA from the N-characteristics continue to be unknown but can hypothetically be related to the specific features of microbial and plant economics in various experimental variants.

## CONCLUSIONS

The expediency of argumentation of changes in N-, C-chemical, N-biological soil indicators ( $N_{IN}$ ,  $N_{EH}$ ,  $C_{LH}$ , *in situ* PA – isPA, *in vitro* UA – ivUA) in the context of the following hypothetical and theoretically predictable processes in soil is illustrated: *(i)* N-, C-mineralisation/immobilisation, *(ii)* sequestration, *(iii)* priming, *(vi)* aspects of resource allocation. The most effective were the rationales built in the areas *(i)*, *(iii)*, *(vi)*. The statistical validity of the predetermination of grain weight per sown area of GM winter wheat by the interdependencies  $N_{IN}$ ,  $N_{EH}$ , isPA was proved. The simultaneous significant probability of the interrelationships between  $C_{LH}$  and ivUA revealed the disconnect between the availability and demand of microorganisms for C and N under these experimental conditions. Among the crucial properties of the causal relationships between these variables were the stimulating effect of  $N_{IN}$ ,  $N_{EH}$ , and the reverse effect of isPA on GM, as well as a more significant mediation of isPA action through  $N_{IN}$ ,  $N_{EH}$  (especially when  $N_{IN}$  is predetermined by isPA level, and at the same time,  $N_{EH}$  size is predetermined by the ratio of isPA and  $N_{IN}$ ), compared to the mediation of the proportion of  $N_{IN}$ ,  $N_{EH}$  effects through isPA (considering the predetermination of  $N_{IN}$  by  $N_{EH}$  size or *vice versa*). In contrast to the first of the above variants of variable premixing, the second helped to partially isolate the elements of the basis for the opposite influence of  $N_{IN}$ ,  $N_{EH}$ , on the one hand, and isPA, on the other hand, on GM formation. The non-causal conditioning of  $C_{LH}$  by ivUA did not take part in the development of GM variation but was probably associated with the effect of unidentified factor variables. The complex structure of the data (deviations from normal distribution, multicollinearity, etc.) and the presence of unidentified predictors herein, hinder the search for reliable simple, yet extensive mathematical formalisations for causality or directionality of relationships between variables. Prioritising combinations of N-, C-chemical, and biological soil indicators, identifying and analysing potential and/or actual causal relationships between them and crop productivity traits is vital for constructing minimum sets of parameters and indices of soil health and quality relevant for predicting potential and actual soil fertility. In the future, it is significant to search for and analyse, using statistical/machine learning methods, supplementary features to those presented herein that may contribute to the formation of the C/N balance in soil

and crop bioproductivity, particularly under sustainable farming technologies.

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## CONFLICT OF INTEREST

The authors of this study declare no conflict of interest.

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## Каузальні залежності між продуктивністю пшениці й біологічною активністю, вмістом у ґрунті азоту

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**Анотація.** Висвітлення компонентів каузальних залежностей продуктивності сільськогосподарських культур, опосередкованих N-, C-хімічними, біологічними індикаторами ґрунту (розміри пулів відповідних доступних форм, ферментативних активностей) у контексті теоретичних аспектів обігу ресурсів у ньому, є потенційно важливим для конструювання систем оціночних критеріїв здоров'я ґрунту з використанням моделей машинного навчання (ML). Мета роботи полягала у відшукуванні статистично валідних і змістовних координацій і каузальних зумовленостей маси зерна на площу посіву доступними формами N, C (неорганічний і легкогідролізований N – N\_IN, N\_EH; C лабільного гумусу – C\_LH), *in situ* протеолітичною PA, *in vitro* уреазною UA активністю у асоціації з концепціями обігу зазначених органогенів у ґрунті під пшеницею озимою за прототипних екологічно безпечних систем добрив. Використано наступні методи: польові, аплікаційний (*in situ*), лабораторно-аналітичні хімічні й біохімічні, морфометричний, статистичні, ML-моделювання. З високою ймовірністю приріст GM був спричинений адитивним зростанням N\_IN, N\_EH, одночасним зменшенням *in situ* PA у спосіб, за якого вплив *in situ* PA на GM опосередковувався переліченими формами N. Статистично менш валідним було опосередкування дії співвідношення N\_EH до N\_IN на GM через *in situ* PA з, можливо, взаємно доповнюючими предетермінуваннями форм N, що втім, створювало основу для обґрунтування одночасних взаємно протилежних зумовленостей GM цими формами N й *in situ* PA. Правдоподібно, що C\_LH й *in vitro* UA не брали участі у розвитку GM за даних експериментальних умов, проте не виключено, що друга серед цих змінних може більш значуще впливати на варіювання першої, ніж навпаки. Дослідження у такому руслі дозволять сформулювати дискурс теоретичних передбачень стосовно удосконалення структури індексів якості ґрунту у напрямку розкриття аспектів закономірностей сприяння необхідному для розвитку стійкого сільського господарства балансу між характеристиками агроєкосистемних послуг, з урахуванням хімічних і біохімічних властивостей обігу N і C у ґрунті, й біопродуктивності сільськогосподарських культур

**Ключові слова:** доступні форми азоту у ґрунті; вуглець лабільного гумусу; протеолітична й уреазна активність ґрунту; маса зерна пшениці озимої; експлораторний факторний аналіз; системи одночасних рівнянь; каузальні зумовленості

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