









Article

Effects of Sewage Sludge Compost on Carbon, Nitrogen, Phosphorus, and Sulfur Ratios and Soil Enzyme Activities in a Long-Term Experiment

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Abstract: The carbon, nitrogen, phosphorus, and sulfur (CNPS) ratios of soils are known to be relatively stable parameters, characterizing different land uses. We hypothesized that the long-term application of sewage sludge compost (SSC) would not change these ratios but would increase the concentration of these elements and change the quality of organic matter (OM), as well as soil enzyme activities. Hence, soil chemical and microbiological properties were studied in a 20-year long-term experiment. The plots were grouped into five blocks and treated every third year with SSC at the rates of 0, 9, 18, or 27 t ha⁻¹. Three plants, in a crop rotation, were tested and sown every year as follows: rye, rye with hairy vetch, and maize. The results showed that basic soil parameters (pH, OM content, E4/E6 ratio, NO₃-NO₂-N, AL-P₂O₅, and soil moisture content) were increased, along with the SSC doses in soil for the rye. Similar trends were found in CNPS concentrations, β-glucosidase, and alkaline phosphatase activities, while the acidic phosphatase activity was reduced. The C:N, C:S, and N:S ratios were not affected by the compost application. The main factors of treatment effects were plant-available phosphorus (ammonium lactate (AL)-soluble P₂O₅), total P, and NO₃-NO₂-N, based on principal component analysis. The canonical correspondent analyses revealed that phosphatase activities were affected by C:N, C:P, and N:P ratios and β-glucosidase was correlated with P forms and the E4/E6 ratio, while the soil pH strongly affected all soil enzymes. Based on the alkaline and acidic phosphatase activities, the role of microbes became more important with increasing compost doses in phosphorus mobilization. We conclude that the addition of SSC could improve soil health through increasing the pH, OM, nutrient content, and microbial activity. Also, some elemental ratios have an important role in the regulation of soil enzyme activities.

Keywords: sandy soil; organic matter; waste utilization; CNPS ratios; glucosidase; phosphatases



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1. Introduction

All the elements that make up living organisms are participants in the biogeochemical cycle [1]. The appropriate ratio of the elements affects the availability of soil nutrients, plant

growth, and microbial processes [2]. Ecological stoichiometry is an important indicator of the changes taking place in the soil, which affect not only the soil but also human health, crop production, and biodiversity. It reflects the mineralization or immobilization processes in the soil. The soil C:N ratio characterizes the mineralization of organic matter, while the C:P ratio characterizes phosphorus availability [3]. Moreover, a significant correlation is the C:N:P, especially the N:P stoichiometric relationship between soils and plants [4].

Different ecosystems or soil depth can be described by varying C:N:P ratios [5]. The C:N:P stoichiometric value applicable to microbial biomass is 60:7:1 and that applicable to plant litter is about 3000:46:1, while that applicable to enzymatic acquisition activities is 1:1:1 [6,7]. Enzymatic stoichiometry is one approach to evaluating the relationship between microbial nutrient demand and nutrient availability [8]. The C:N:P ratio of agricultural ecosystems (64:5:1) is much lower compared to that of natural ecosystems, which is between 287:17:1 and 186:13:1 [9], which may be related to the use of P fertilizers [10] affecting microbial growth and diversity [11]. In their study, Xu et al. [12] (2024) pointed out that warming as a climatic change affects the C:N:P stoichiometry of soil, microorganisms, and plants, which affects P deficiency. In another study, numerous locations were examined around the world, including surface and sub-surface soils of agricultural, pasture, and unfarmed lands [13]. They found that the C:N ratio could be in the range of 9.9–17.5 and that the C:P ratio might vary from 44 to 287, while they found the C:S ratio to be between 54 and 132 in soils. A study on humus status over 27 years in natural and agricultural Arenosols [14] demonstrated that croplands with fertilizers and without fertilizers had the lowest C:N ratio (14.7–15.5) compared to other Arenosols (15.8–19.8), like fertilized and non-fertilized cut grasslands, abandoned land, and pine afforestation fields. Several land uses have different effects on soil C sequestration and the quality of soil organic matter (SOM). The influence of different land uses and ecosystems on soil carbon sink capacity and microbial functional diversity was also confirmed in a study by Lai et al. [15]. Moreover, many authors have pointed out that climate changes have a strong influence on the C:N ratio in soil. Immobilization and N availability are closely linked and determine the soil carbon stock [16,17]. An increase in soil N content results in high microbial carbon utilization efficiency (CUE) and less C loss through microbial respiration, while low N availability in soil results in low microbial CUE and increased microbial respiration [18]. Rising anthropogenic chemical releases influence the N:P ratio. An increase in the N:P ratio of soil, water, and plants can be observed on a global level, yet P application to cropland soils reduces the N:P ratio [19].

A correlation was found between the P concentration of soil and the S-acquiring enzyme activity, whereby the S concentration of the soil had a negative impact on soil S-related enzyme activity [20].

Microbes play a major role in the global biogeochemical cycle and carbon storage. Supplying the soil with organic matter has a favorable effect on the growth and activity of the microbial communities involved in the nutrient fluxes [21]. Through the activation of microorganisms, soil amendments containing organic matter play an important role in the formation of the stoichiometry of agricultural soils, especially regarding P turnover [22].

Besides the presence of nutrients, the way in which they are mobilized is one of the main factors in plant nutrient uptake. The physical, chemical, and microbiological properties of soils affect nutrient availability in different combinations and with different degrees of effectiveness [23,24]. Organic matter-bound nutrients are made available to plants by microorganisms during their mineralization processes, where the microorganisms are also meeting their own stoichiometric needs [25]. The soil is the largest C reservoir, and its humic substances represent the stable parts of the geochemical cycle of C in the soil over the long term [26]. In soils with a high SOC content, N and P are the limiting factors in

microbial biomass synthesis and metabolism [27]. Soil enzymes play important roles in the mobilization of soil C, N, P, and S elements.

Glucosidase enzyme is related to the carbon cycle and soil organic matter, providing an easy-to-use carbon source for microorganisms [28]. Several studies have reported that the use of soil organic amendments increases the activity of this enzyme as a consequence of the growing microbial population and activity [29,30].

The mineralization of organic P forms in the soil takes place besides phytases and CP-lyases with the help of phosphomonoesterases produced by plants and soil microorganisms [31]. The phosphatase and glucosidase enzyme activities in an agricultural Mediterranean area treated with sewage sludge increased due to the enhanced native microbial community and/or the new microbial biomass and to its content of intra- and extracellular enzymes [32]. The response of plants, bacteria, and fungi to inorganic P deficiency is the secretion of phosphatases [33]. An increasing inorganic P concentration does not necessarily cause a decrease in acidic phosphatase (ACP) enzyme activity, because the P retention capacity of different soil types can also be an influencing factor [34]. Therefore, the results proved that available P from inorganic P-fertilizers reduced ACP activities while slightly increasing ALP activities, depending on ecosystem types and fertilization management [35], and a direct correlation was also found between soil P availability and phosphatase enzyme activity [36]. The available P content of the soil is one of the limiting factors in plant growth. Organic phosphorus must be mineralized first to make it available to plants [37]. It is a well-known fact that the main influencing factor in enzyme activities is the amount of available P substrate [38] and the P requirement of microorganisms and plants [39].

Sewage sludge is a source of OM, which could be utilized in degraded soils. In Szabolcs–Szatmár–Bereg County, Hungary, about 9000 tons (dry matter) of sludge is generated yearly, with 75–80% organic matter content. The second-largest territory of sandy soils in Hungary is in the Nyírség region of this county, where the addition of OM to the soil, especially in plowed lands, is essential. Composting and utilizing sewage sludge in arable lands fits with the principles of the European Green Deal and Circular Economy. The ratio of the macro-elements in sewage sludge compost (SSC) depends on the applied structural materials and other additives. The C:N ratio of straw, which is the organic additive in our SSC, is 80–100 [40]. Moreover, the applied additives could modify the quality of the OM in the composts [41], from which the stored macro-, meso- and microelements are altered to be bioavailable by the microbial community of soils [42]. The application of SSC generally increases the enzyme activities of soils [43,44] and causes a shift in the soil microbial community [45], but a high dose of SSC could reduce the enzyme activity of treated soil [46].

According to the literature, the C:N:P:S ratios of soils and microbial communities are relatively stable. Therefore, we hypothesized that the regular application of SSC would not change the soil C:N:P:S ratios, but the changes in the absolute C, N, P, and S concentrations would affect the microbial activity of treated soils. Therefore, the objectives of this work were to (i) study the CNPS ratios of sandy soil after the long-term application of sewage sludge compost, (ii) measure the quantity and quality of soil organic matter, and (iii) identify the main environmental factors affecting the measured soil enzymes.

2. Materials and Methods

2.1. Experimental Site and Soil Sampling

The long-term sewage sludge compost (SSC) experiment was established in 2003 and modified in 2006 in the experimental field of the Research Institute of Nyíregyháza, IAREF, University of Debrecen, Hungary. The basic information about the soil, climate, crops, and design periods in this long-term experiment can be found on Global Long-Term

Agricultural Experiment Network of Rothamsted Research “://glten.org/experiments/166 (08.01.2025)”. The geographical location of the experiment is at latitude 47°98.8273' N, longitude 21°70.362' E, altitude 106 m a.s.l.

According to the WRB, the soil type is Dystric Arenosol, Aric, and Cordic [47] and the physical texture is 87.69% sand, 2.67% silt, and 9.64% clay. The main chemical properties of the experimental site at the beginning of the experiment were as follows: Ph (KCl), 5.38; SOM, 0.92%; total N, 0.079%; plant-available ammonium lactate (AL)-soluble P₂O₅, 239.0 mg kg⁻¹; and AL-K₂O, 183.8 mg kg⁻¹. This was a non-randomized plot (12 m × 19 m) experiment, where the 0, 9, 18, and 27 t ha⁻¹ SSC treatments were grouped into 5 blocks, which meant 5 replications of each treatment, because the field experiment design was the same in all blocks (Figure 1). The applied SSC was designed for acidic sandy soils, containing 40% (m/m) of sewage sludge, 25% (m/m) of straw, 30% (m/m) of rhyolite, and 5% (m/m) of bentonite, and it was plowed to the 0–25 cm soil layer every third year. The control plots never received SSC nor chemical fertilizer, and the SSC-treated plots never received chemical fertilizer. The last SSC application was performed in 2021 when the main parameters of the applied compost were as follows: pH, 6.9; total organic matter, 24.6%; total nitrogen, 16,000 mg kg⁻¹ (dry matter, d.m.); total phosphorus, 26,800 mg P₂O₅ kg⁻¹ (d.m.); total potassium, 3994 mg K₂O kg⁻¹ (d.m.); calcium, 7900 mg kg⁻¹ (d.m.); and magnesium, 1302 mg kg⁻¹ (d.m.). The SSC met the requirements of Hungarian and European Union regulations based on the concentrations of toxic elements, which were under the limit.

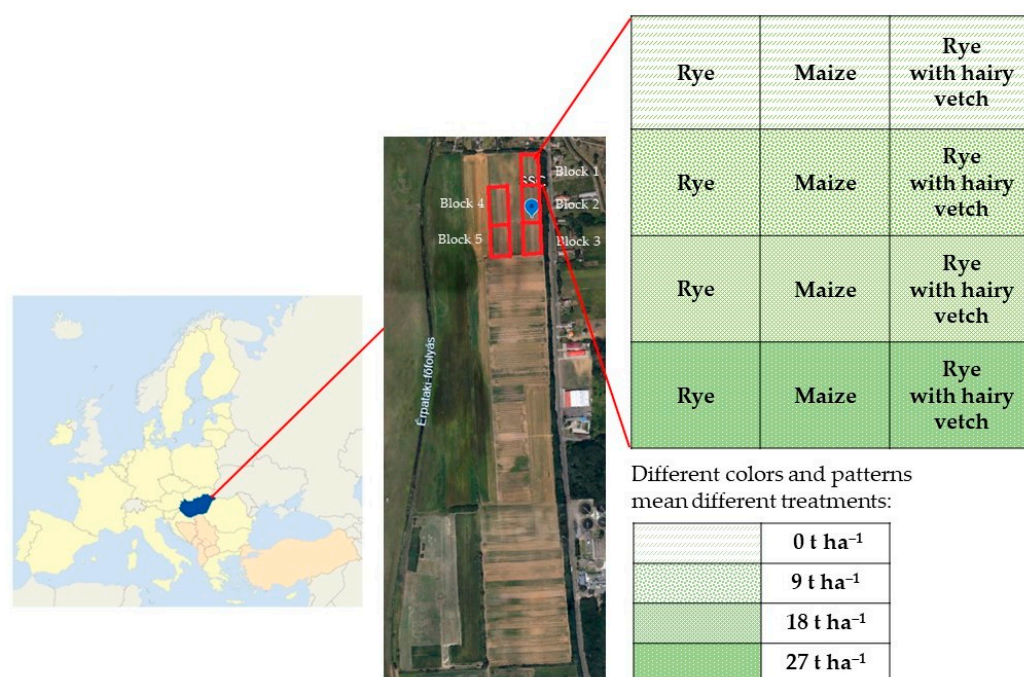


Figure 1. Location of study area, crop rotation (2023), and sewage sludge compost doses in the long-term sewage sludge compost experiment in Nyíregyháza, Hungary. The five blocks on the figure mean five replications. On the right, the treatments and plants in one block are detailed.

Three test plants as a crop rotation system were sown every year, as follows: rye (*Secale cereale* ‘Varda’), rye with hairy vetch (*Vicia villosa* ‘Hungvillosa’), and maize (*Zea mays* ‘Torino’). This was a non-randomized split-plot experiment where the main factor was the SSC treatment, which was split into three plants. The rye and hairy vetch varieties were produced by Research Institute of Nyíregyháza, while the maize ‘Torino’ was the variety Syngenta Global AG, with the FAO number 340–360. To measure the rye yield, plants were

collected from $4 \times 1 \text{ m}^2$ of each plot. The measured yields were calculated in tons per hectare, and the average values of the different plots were used for the statistical analysis.

Composite soil samples from the 0–20 cm soil layer of the rye plots were collected on 6 September 2023, two years after the last SSC treatment. Five subsamples were collected from each plot with an auger and mixed thoroughly. This composite sample was used for physical, chemical, and microbiological analysis. Because there were five blocks on the experimental field, we collected five composite samples from each treatment, resulting in five replications of samples. Samples for soil chemical analysis were air-dried, sieved ($\text{\O} 2 \text{ mm}$), and stored. For measurements of enzyme activities, soil samples were carried to the laboratory in cooling boxes and sieved ($\text{\O} 2 \text{ mm}$), the remaining plant residues were picked up by forceps, and the prepared samples were stored at $-20 \text{ }^\circ\text{C}$ until the measurements were taken.

2.2. Soil Chemical Analysis and Moisture Content Measurement

Soil pH was measured in 1:2.5 soil–1M KCl suspension [48]. Soil organic matter (SOM) content was measured according to the Tyurin method [48]. The plant-available ammonium lactate (AL)-soluble P_2O_5 was colorimetrically measured according to the Hungarian Standard [49]. The $\text{NO}_3\text{-NO}_2\text{-N}$ content was determined according to the Griess–Ilosvay reaction [50]. Total P was measured with ICP-AES after extracting P with 1 mol/L HCl [51]. The E4/E6 ratio was measured at wavelengths of 465 and 665 nm in NaOH extraction [52]. The total carbon (C), nitrogen (N), and sulfur (S) of soil were measured with a VarioMax CNS analyzer (Elementar GmbH, Langenselbold, Germany), according to the Dumas dry combustion method [53]. Soil moisture content was gravimetrically measured by drying the samples at $105 \text{ }^\circ\text{C}$ [48].

2.3. Soil Enzyme Activity Measurement

Three enzymes were selected based on their roles in the C cycle (β -glucosidase) and P cycle (acid phosphatase and alkaline phosphatase). β -glucosidase (GLUC) activity was measured according to Eivazi and Tabatabai [54]. Acidic phosphatase (ACP) and alkaline phosphatase (ALP) activity were determined based on the method of Eivazi and Tabatabai [55]. Briefly, the appropriate substrates, *p*-nitrophenyl- β -D-glucoside for GLUC and *p*-nitrophenyl phosphate for ACP and ALP, were added to one gram of soil and incubated at defined pH values for one hour at $37 \text{ }^\circ\text{C}$, and the produced *p*-nitrophenol (PNP) was measured using a UV-6300PC Spectrophotometer (VWR International Ltd., Debrecen, Hungary) at the wavelength of 405 nm. The enzyme activities were expressed in $\mu\text{g PNP} \cdot \text{g}^{-1} \text{ dry soil} \cdot \text{hour}^{-1}$.

2.4. Statistical Analysis

Microsoft Excel 2023 software was used for datasheet preparation. First, the datasheet was analyzed for the external values, which were removed from the tables before the start of the statistical evaluations. Then, the treatments were compared with one-way ANOSIM using Past software v4.03 to compare the similarity and/or difference between the treatments. One-way analysis of variance (ANOVA) was used to study the treatment's effect on the studied parameters, while the means were compared by Tukey's test. The relationship between soil chemical properties and enzyme activities was studied by correlation analysis. The ANOVA, Tukey's test, and the correlation analysis were performed by using IBM SPSS v28.0.1.0. Principal component analysis (PCA) was used to test the chemical properties of treated soils. To study the effects of environmental factors on the studied enzyme activities, canonical correspondence analysis (CCA) was carried out. Both PCA and CCA were performed using Past software v4.03. All the statistical methods were carried out at a 0.05 significance level.

2.5. Quality Control and Assurance

All reagents used for the calibration of equipment were purchased from Merck Life Science Ltd., Budapest, Hungary. By repeating the testing of the samples five times (repeatability test), the analytical methods precision was determined and was represented as the standard deviation (SD). The average recovery of the CNS analyzer was >99.5% in the case of standard samples. The accuracy of the total C, N and S was verified by calibrating the equipment with a certified reference material (Sulfadiazine, C₁₀H₁₀N₄O₂S). The pH electrode was calibrated by two standard buffer solutions at 20 °C: C₆H₈O₇/NaOH/HCl (pH 4) and Na₂HPO₄/KH₂PO₄ (pH 7), both of them directly traceable to primary SRM from NIST/PTB. The accuracy of the spectrophotometer was verified for the enzyme activity assay by the calibration curves of the standard solution (*p*-nitrophenol for the measured enzymes). For method validation, all the analyses in the study were compared with a laboratory control sample (LCS).

3. Results

3.1. Chemical Properties of the Soil

To compare the treatments based on the measured soil chemical properties, the ANOSIM method was used (Table 1). The results showed that after 20 years, all the treatments were different from each other based on all the measured chemical parameters.

Table 1. Results of ANOSIM statistical analysis of chemical data (pH, SOM, E4/E6, NO₃-NO₂-N, AL-P₂O₅, total C, N, S, and P, and C:N, C:S, C:P, N:P, N:S, and P:S ratios) and moisture content obtained from the 0–20 cm soil layer in a 20-year old sewage sludge long-term experiment. *p* < 0.0001.

	0 t ha ⁻¹ SSC	9 t ha ⁻¹ SSC	18 t ha ⁻¹ SSC	27 t ha ⁻¹ SSC
0 t ha ⁻¹ SSC	–	0.0084	0.0086	0.0082
9 t ha ⁻¹ SSC	0.0084	–	0.0072	0.0075
18 t ha ⁻¹ SSC	0.0086	0.0072	–	0.0626
27 t ha ⁻¹ SSC	0.0082	0.0075	0.0626	–

After 20 years of the experiment, some chemical parameters were significantly changed in the treatment plots (Table 2). The soil pH increased significantly and reached 6.16 in the plots with the highest SSC dose, where the SOM content was also the highest, with a value of 0.85%, which is a particularly important change in soil with low buffering capacity. The values of SOM increased by 14.5%, 21%, and 37.1% in the treatments compared to the control plots. The quality of SOM was not different in the treatments based on the E4/E6 value, but the slightly increasing E4/E6 values of the treated plots indicated that the SSC contained younger organic matter (OM) than the soil, which was less resistant to mineralization.

However, the NO₃-NO₂-N values were higher in the treated plots, compared to the control ones, but the changes were not significant. Despite the lack of significance, these results reflect well the effect of the applied SSC on soil-available N content with increasing NO₃-NO₂-N values with the treatments (3.6, 37.2, and 18.4%, respectively). On the other hand, the AL-P₂O₅ content changed significantly along with the increasing doses of the applied SSC, ranging from 106.36 (control) to 696.40 mg kg⁻¹ (27 t ha⁻¹), demonstrating that SSC can be used as a good source of P in long-term application. The values of AL-P₂O₅ of the plots treated with the highest doses indicated a more than adequate available P level for plants to grow. The soil moisture content was higher by 10.3% in the plots of the highest SSC treatment, but this increase was not proven statistically. On the other hand, it can be stated that the soil moisture content increased with increasing SOM content.

Table 2. Chemical properties and moisture content (mean \pm standard deviation) of the 0–20 cm soil layer, regularly treated with different doses of sewage sludge compost (SSC). Different letters indicate statistical difference in means according to Tukey’s test ($p < 0.05$).

Parameter	0 t ha ⁻¹ SSC	9 t ha ⁻¹ SSC	18 t ha ⁻¹ SSC	27 t ha ⁻¹ SSC
pH	4.38 \pm 0.47a	5.37 \pm 0.60b	5.94 \pm 0.58b	6.16 \pm 0.32b
SOM (%)	0.62 \pm 0.01a	0.71 \pm 0.09ab	0.75 \pm 0.04ab	0.85 \pm 0.11b
E4/E6	6.67 \pm 0.78a	7.30 \pm 0.53a	7.50 \pm 0.26a	7.66 \pm 0.26a
NO ₃ -NO ₂ -N (mg kg ⁻¹)	14.53 \pm 1.59a	15.05 \pm 1.65a	19.94 \pm 6.44a	17.20 \pm 4.96a
AL-P ₂ O ₅ (mg kg ⁻¹)	106.36 \pm 49.32a	259.75 \pm 7.89a	459.75 \pm 29.51b	696.40 \pm 143.71c
Total C (mmol kg ⁻¹)	388.224 \pm 18.223a	429.050 \pm 7.839ab	471.443 \pm 21.545b	558.204 \pm 53.069c
Total N (mmol kg ⁻¹)	39.823 \pm 1.008a	41.914 \pm 3.323ab	47.790 \pm 0.450bc	52.784 \pm 5.299c
Total P (mmol kg ⁻¹)	8.546 \pm 0.783a	12.858 \pm 1.063a	18.356 \pm 2.277b	21.044 \pm 4.273b
Total S (mmol kg ⁻¹)	3.000 \pm 0.191a	3.225 \pm 0.081ab	3.653 \pm 0.270bc	4.102 \pm 0.383c
Moisture (% <i>m/m</i>)	8.55 \pm 0.11a	8.78 \pm 1.06a	7.94 \pm 0.76a	9.43 \pm 0.82a

The applied sewage sludge compost is a complex material of organic and inorganic components containing both macro- and microelements. In this study, the long-term application of the SSC resulted in a 10.5–43.8% increase in total C content in the sandy soil, while the total N content increased by 5.3–32.5% and the total P content increased by 50.5–146.2% with the increasing compost doses (Table 2). The increasing rate of the total S content was similar to N, with 7.5–36.7% compared to the control treatment.

Regarding the ratios of the studied elements in the SSC-treated soil (Table 3), the changes in C:N, C:S, and N:S ratios were not proven statistically; however, the C:N ratio was higher in the treated plots than in the control ones, while the tendency was the opposite in the case of the N:S ratio. The values of the C:S ratio were almost similar in the 0, 9 and 18 t ha⁻¹ treatments (129.50, 127.36, and 127.46, respectively), while it was slightly higher (136.37) in the highest SSC treatment.

Table 3. Ratios of total CNPS values (mean \pm standard deviation) of the 0–20 cm soil layer regularly treated with different doses of sewage sludge compost (SSC). Different letters indicate statistical differences in means according to Tukey’s test ($p < 0.05$).

Parameter	0 t ha ⁻¹ SSC	9 t ha ⁻¹ SSC	18 t ha ⁻¹ SSC	27 t ha ⁻¹ SSC
C:N	9.968 \pm 0.327a	10.156 \pm 0.318a	10.068 \pm 0.131a	10.455 \pm 0.154a
C:P	47.375 \pm 2.238c	31.783 \pm 1.220b	28.720 \pm 1.714ab	24.375 \pm 2.86a
C:S	129.502 \pm 5.183a	127.366 \pm 11.006a	127.456 \pm 10.193a	136.372 \pm 11.078a
N:P	4.805 \pm 0.294c	3.177 \pm 0.061b	2.883 \pm 0.196b	2.335 \pm 0.288a
N:S	12.982 \pm 0.734a	12.370 \pm 0.197a	12.383 \pm 0.443a	12.876 \pm 0.822a
P:S	2.755 \pm 0.101a	3.842 \pm 0.249b	4.752 \pm 0.793bc	5.280 \pm 0.165c

The ratio of C:P elements significantly decreased with the increasing SSC doses from 47.38 to 24.38. Similar results were found in the case of the N:P ratios, where the values decreased from 4.81 to 2.34 with the increasing SSC doses. On the other hand, the P:S ratio increased with increasing doses of compost. The 2.76 P:S ratio measured in the control treatment increased up to 5.28 in value in the plots treated with 27 t ha⁻¹ SSC.

3.2. Soil Enzyme Activities

The activity of hydrolytic soil enzymes, such as β -glucosidase and acid and alkaline phosphatase, involved in C and P cycles were measured in our study.

Glucosidase and alkaline phosphatase activities increased with the increasing doses of SSC added to the soil. All of the three applied SSC doses had a significant effect on the activities of these soil enzymes. The activity of β -glucosidase increased by 167.13%, 209.09%, and 236.50%, respectively, in the treatments compared to the control soil. The

increase in ALP activity along with SSC doses was higher than in the case of β -glucosidase by the rates of 249.13%, 397.42%, and 476.49%, respectively. The acidic phosphatase activity decreased with increasing SSC doses, but the changes were not significant, and its activity was much higher, especially in the control plots, than the activity of alkaline phosphatase (Table 4). The ACP activity decreased by 1.68%, 27.87%, and 13.91% in the 9, 18, and 27 t ha⁻¹ SSC treatments, respectively, compared to the control ACP activity.

Table 4. Soil enzyme activities and their ratios (mean \pm standard deviation) in the 0–20 cm soil layer regularly treated with different doses of sewage sludge compost (SSC). Different letters indicate statistical difference in means according to Tukey's test ($p < 0.05$).

Parameter	0 t ha ⁻¹ SSC	9 t ha ⁻¹ SSC	18 t ha ⁻¹ SSC	27 t ha ⁻¹ SSC
Glucosidase ($\mu\text{g PNP g}^{-1} \text{hr}^{-1}$)	39.715 \pm 14.911a	66.375 \pm 7.251b	83.041 \pm 6.779bc	93.926 \pm 92.817c
Acidic phosphatase ($\mu\text{g PNP g}^{-1} \text{hr}^{-1}$)	448.301 \pm 68.330a	440.787 \pm 195.797a	323.369 \pm 99.263a	385.936 \pm 130.754a
Alkaline phosphatase ($\mu\text{g PNP g}^{-1} \text{hr}^{-1}$)	60.773 \pm 8.079a	151.404 \pm 50.506b	241.525 \pm 59.081c	289.576 \pm 48.266c
lnGLUC/lnACP	0.597 \pm 0.031a	0.700 \pm 0.028b	0.750 \pm 0.025b	0.768 \pm 0.010b
lnGLUC/lnALP	0.843 \pm 0.021a	0.847 \pm 0.022a	0.810 \pm 0.021a	0.789 \pm 0.009a
lnGLUC/ln(ACP + ALP)	0.579 \pm 0.025a	0.660 \pm 0.018b	0.683 \pm 0.002b	0.698 \pm 0.014b

We found that the effects of sewage sludge compost on C- and P-acquiring enzymatic activity ratios varied with the applied doses. Looking at the sum of the ACP and ALP activities, an increase can be seen with the treatments, indicating higher P-enzyme investment. The increase in ACP + ALP activities may be a response to higher microbial and plant demands to cover their phosphorus needs.

Higher C and P cycle-related enzyme activity was found with higher sewage sludge compost application rates. The lnGLUC and lnACP enzyme ratio ranged between 0.60 and 0.77, increasing with the applied doses, while lnGLUC/lnALP decreased, ranging between 0.84 and 0.79. Comparing the investments to C- and P-acquiring enzymes using the equation lnGLUC/ln(ACP + ALP), we can state that the investment of microbes into the C-acquiring enzyme was higher than that of P-acquiring ACP and ALP together, indicated by the increasing ratios seen with increasing SSC doses (Table 4).

3.3. Evaluation of the Most Effective Soil Chemical Parameters

The regular, long-term application of sewage sludge compost resulted in well-separated treatments according to principal component analysis (PCA) on soil chemical properties (Figure 2). The control and 9 t ha⁻¹ treatments are on the negative side of the PCA graph, while the 18 and 27 t ha⁻¹ SSC treatments are separated on the positive side along with Component 1, which accounts for 83.78% of the total variance, while PC2 determines 7.51% of the total variance. The control treatment is very far from the SSC treatments on PC1, indicating higher differences between the control and treated samples than among the treatments. The main factors in PC1 resulting in differences among treatments are AL-P₂O₅ and Total P, while C:P, N:P, and P:S had smaller effects on PC1. The main factor causing the variability within the treatments for PC2 is NO₃-NO₂-N, but the total C, N, and moisture content also affected the treatments. Based on the PCA results, the pH values had no strong effect on the differentiation in the treatments.

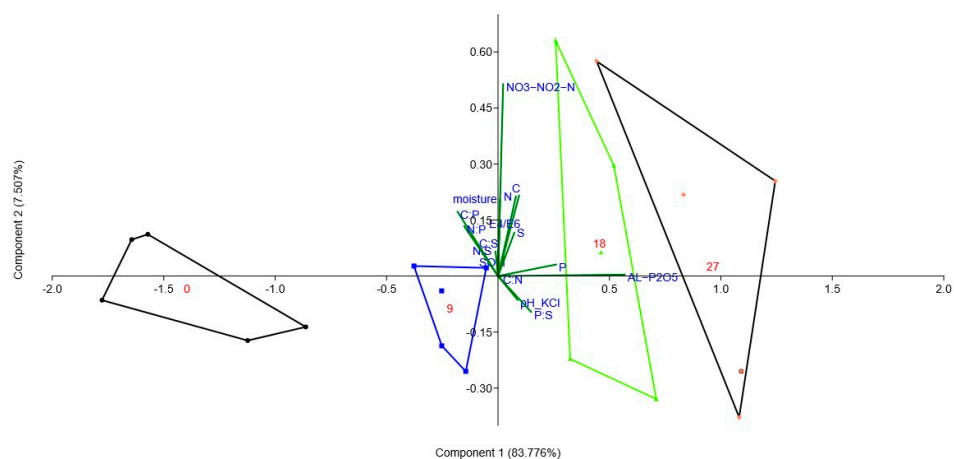


Figure 2. Principal component analysis of the measured soil chemical properties (pH, SOM, E4/E6, NO₃-NO₂-N, AL-P₂O₅, total C, N, S, and P, and C:N, C:S, C:P, N:P, N:S, and P:S ratios) and moisture content in a long-term sewage sludge compost experiment. The *p* value for the ANOSIM analysis of soil chemical properties was 0.0001.

Canonical correspondence analysis (CCA) was used to study the effects of soil moisture content and chemical parameters on the activity of soil enzymes (Figure 3). Although the pH and elemental ratios were not important for discrimination among the SSC treatments according to the PCA results, the CCA indicated their important role in the regulation of soil enzyme activities, which were separated mainly by the pH, AL-P₂O₅, total P, and C:P, N:P, and P:S ratios. Besides these parameters, ALP activity was correlated positively with the C:N and C:S ratios. ACP was negatively correlated with pH and P forms, but a positive relationship was found with the N:P and C:P ratios. β-GLUC positively correlated with pH and P forms, as well as with C, N, and S elements, indicating the role of organic matter in the activity of this enzyme. The quality of organic matter (E4/E6) was more important in the determination of β-GLUC activity than the OM quantity. In the case of long-term SSC application, the roles of the elemental ratios were very important, alongside the elemental contents and other chemical properties, in determining enzyme activities. Out of the studied soil moisture content and chemical parameters, pH had the strongest effect (score: −0.9745) on enzyme activities; β-GLUC and ALP had positive correlations with it, while ACP decreased with increasing pH values.

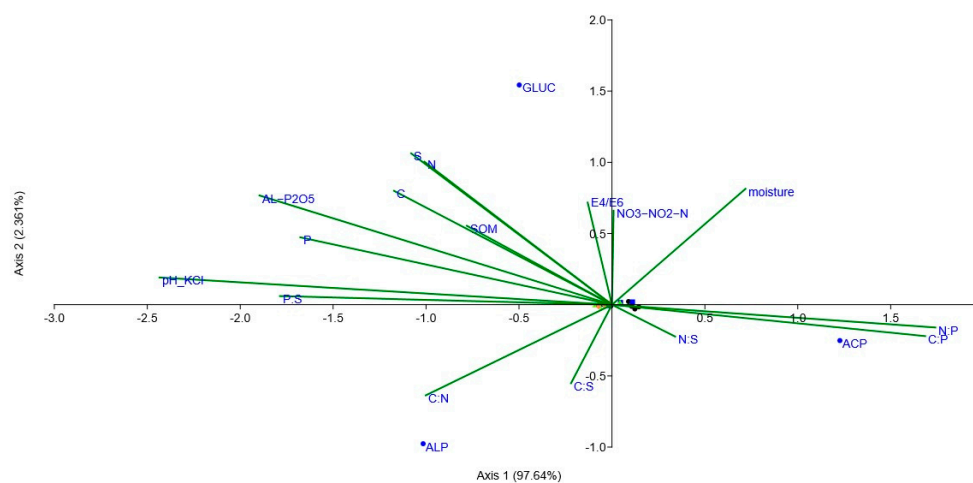


Figure 3. Canonical correspondence analysis of measured chemical properties (pH, SOM, E4/E6, NO₃-NO₂-N, AL-P₂O₅, total C, N, S, and P, and C:N, C:S, C:P, N:P, N:S, and P:S ratios), moisture content, and enzyme activities (β-glucosidase—GLUC, acid phosphatase—ACP, alkaline phosphatase—ALP) in a long-term sewage sludge compost experiment.

The analysis of the relationships between enzyme activities and soil physicochemical properties proved the results of CCA showing strong similarities between GLUC and ALP activities regarding the significant correlations with the same soil physicochemical properties like pH, SOM, E4/E6, AL-P₂O₅, C, N, P, S, C:P, N:P and P:S (Table 5).

Table 5. Correlation matrix between soil physicochemical parameters and enzyme activities (GLUC, ACP, and ALP). *, $p < 0.05$; **, $p < 0.01$; ns, not significant.

	GLUC ($\mu\text{g PNP g}^{-1} \text{hr}^{-1}$)	ACP ($\mu\text{g PNP g}^{-1} \text{hr}^{-1}$)	ALP ($\mu\text{g PNP g}^{-1} \text{hr}^{-1}$)
pH (KCl)	0.815 **	−0.666 **	0.936 **
SOM (%)	0.765 **	ns	0.502 *
NO ₃ -NO ₂ -N (mg/kg)	ns	ns	ns
AL-P ₂ O ₅ (mg/kg)	0.860 **	ns	0.859 **
E4/E6	0.751 **	ns	0.697 **
Moisture %	ns	0.714 **	ns
C (mmol/kg)	0.832 **	ns	0.704 **
N (mmol/kg)	0.788 **	ns	0.621 *
S (mmol/kg)	0.830 **	ns	0.749 **
P (mmol/kg)	0.834 **	ns	0.785 **
C:N	ns	ns	ns
C:S	ns	ns	ns
C:P	−0.812 **	ns	−0.812 **
N:S	ns	ns	ns
N:P	−0.811 **	ns	−0.827 **
P:S	0.770 **	ns	0.836 **

3.4. Grain Yield of Rye

The yield of rye was non-significantly increased with the increasing of the SSC dose from 5.7 to 12.6% (Table 6), indicating the role of improved soil physicochemical properties and enzymatic activities in a small increase in plant yields.

Table 6. Yield of rye in the sewage sludge compost (SSC) long-term experiment in the sampling year 2023. S.D. means standard deviation. Similar letters indicate that there was no statistical difference in means according to Tukey's test ($p < 0.05$).

Treatment	Yield (t ha ^{−1})	S.D.
0 t ha ^{−1} SSC	4.35a	0.26
9 t ha ^{−1} SSC	4.68a	0.36
18 t ha ^{−1} SSC	4.60a	0.35
27 t ha ^{−1} SSC	4.90a	0.53

4. Discussion

The chemical properties and moisture content, CNPS ratios, and soil enzyme activities of acidic sandy soil are presented after 20 years of the regular application of composted municipal sewage sludge designed specifically for use with acidic sandy soil to improve soil health by increasing its organic matter, clay, and nutrient contents.

4.1. Soil Chemical Properties

The fertility of acidic sandy soils is low because of their low OM content and pH. These parameters significantly increased in acidic sandy soil under conventional cultivation when OM containing municipal sewage sludge compost was applied regularly. Soil pH is an important chemical property affecting the availability of nutrients [56], enzyme activities [57], and microbial community composition [58,59]. The applied complex organo-mineral compost increased the acidic (4.38) pH of the sandy soil to around pH 6 in the 18

and 27 t ha⁻¹ treatments (Table 2). This pH range is better for nutrient availability [60]; however, the nutrient availability of plants is a combined result of the effects of the pH on sorption by soils and on plant uptake. The usual effect on soil and roots is different in cases of certain elements [61].

Our results show that regular SSC application increased the OM content of plowed soil from 0.62% to 0.85%, which has great importance for carbon sequestration because one of the most important threats to soil is the loss of OM [62]. The ratio of E4/E6 refers to the quality of the SOM [63]. The calculated E4/E6 ratios being in the upper 6 indicates the presence of a higher portion of fulvic acids in the soil. The soil fulvic acid content, with its acidic functional groups, contributes to the SOM buffering effect [64]. Fulvic acids in SOM compounds are among the most rapidly mineralizing labile carbons, which easily evaporate into the atmosphere as CO₂ during the transformation of humic substances [65]. This labile form of OM is necessary for plant growth; therefore, cropland cultivation generally has a negative effect on soil organic carbon content [14,15]. The small increase in the proportion of fulvic acids in the soils in all treated plots compared to the control samples indicates that the SSC used contains more fulvic than humic acids. To improve the quality of the SSC with a more stable OM content, a longer composting process should be used to increase the carbon sequestration of treated soil. In addition to their effect on the physical, chemical, and biological properties of the soil, humic substances play a significant role in the nutrient supply of plants in many ways. The humus content of OM increases the release of OM compounds by the roots [66].

Nitrogen and phosphorus are among the most important elements in living organisms [67]. In modern agriculture, chemical fertilizers are used to supply plants at the necessary ratios of these elements. The SSC contains these macro-elements in different forms, so good-quality SSC could serve as an alternative to chemical N and P fertilizers [68] by increasing the plant-available forms of these elements in the soil. The SSC used in this experiment was a very good phosphorous source; the available P content of the soil rose six-fold compared to the control.

Sulfur (S) is an important essential macronutrient in protein synthesis and a constituent of chloroplasts, vitamins, and coenzymes. Plant roots take it up as sulfate ions, while leaves can absorb S in gaseous forms such SO₂ and H₂S [69,70]. Despite the important role of S in living organisms, soils generally have a S deficiency [71]; therefore, the significant increase in soil total S content as a result of higher doses (18 and 27 t ha⁻¹) of the applied SSC represents a favorable effect for plant nutrient supply and for microbes. Sulfur has great importance among plant nutrients because only plants are capable of reducing sulfate and producing S-containing amino acids [72].

4.2. Enzyme Activities

Enzyme activities are informative indicators of soil nutrient dynamics and are complementary parts of conventional soil tests when evaluating nutrient availability. Even though microbial biomass represents only a small fraction of SOM, it has great importance in the nutrient cycle [73]. The ability of soil microbes to degrade macromolecular substrates through enzyme production makes microbes the main drivers of nutrient turnover and contributes to OM dynamics in soils [74]. Enzyme activity measurements can be indicators not only of nutrient availability but also of nutrient limitation in soils [75–77].

The ability of soil microorganisms to secrete extracellular enzymes such as acid or alkaline phosphatase or β -glucosidase enables them to survive in nutrient-limiting environments and control microbial and plant growth efficiency. The enzymes make nutrients available from SOM not only for microbes but also for plants, covering their stoichiometric needs [78]. The β -glucosidase activity of the soil increases as a result of soil amendments

containing easily degradable OM, like the applied SSC, while soils with higher recalcitrant C have lower glucosidase activity [29]. This statement is in accordance with our findings, as a lower E4/E6 ratio indicated more stable OM with lower β -glucosidase activity in the control than in the treated plots (Tables 2 and 4).

The origin of ACP and of ALP is different. Acid phosphomonoesterases (ACPs) are produced by plants and microorganisms [79], while alkaline phosphomonoesterases (ALPs) are mainly the products of microorganisms [80]. Our results revealed that, along with increasing SSC doses increasing the pH, the role of microbes in P solubilization became more and more important, as the increasing ALP and decreasing ACP activities indicate (Table 4). Our results show that ALP activity correlates positively with the SOM and AL-P₂O₅ content of soil; however, Santos-Beneit found that genes encoding ALP were induced by inorganic P limitation rather than the organic P level [81]. The situation is more complex because the C:P, N:P, and C:N ratios also affect ALP and ACP activities [82–84]. Increased ACP and ALP activities are well correlated with an increase in plant biomass and the SPAD index [85], indicating a closed relationship between plant development and microbial activity.

According to the resource allocation theory, C and N added to the soil cause P deficiency due to increased microbial populations, which induces the production of P-releasing enzymes, like acid or alkaline phosphomonoesterases [86]. This was proven by [87], who found that the addition of mineral N reduced ACP enzyme activity in a larch plantation through reducing the labile inorganic P concentration. Adding our studied SSC to the soil with high available P content could eliminate this problem, as proven by the high level of AL-P₂O₅ of treated soils (Table 2). In our long-term experiment, we did not find the opposite effect; both elements (N and P) increased the activity of ALP. Perhaps, the forms of added N and P could be an important factor in these effects.

The ratios of GLUC, ALP, and ACP enzymes are related to the higher C mineralization in treated plots compared to P mineralization. Increasing ratios of C- and P-related enzyme activities indicate a higher investment in C-enzymes. Organic P conversion (represented by ACP and ALP), expressed as the ratio of C-acquired enzyme and phosphatase activities ($\ln\text{GLUC}/\ln(\text{ACP} + \text{ALP})$), was higher in the SSC-treated plots than the control plots (Table 4), representing a decrease in P limitation [88]. Moreover, the increasing ratios of $\ln\text{GLUC}/\ln(\text{ACP} + \text{ALP})$ indicated a higher microbial investment in C-acquiring enzyme activity.

When evaluating soil biological parameters, it should be considered that due to the dynamism of microbial communities, their composition and activity always reflect the current state of the environment [89]. However, in long-term experiments, the cumulative effects of regular treatments could reduce the variability in microbial parameters as a function of the changing environment.

4.3. CNPS Contents and Ratios in Soil

There are important relationships between N, P, and S utilization by plants. Studies have reported a negative interaction between SO₄ uptake of plants and soil N deficiency. N supply influences the gene regulation responsible for S absorption. Nutrition in plants is also influenced by the C turnover of the soils; e.g., the sugar released from organic C compounds enhances the absorption of sulfate ions by plants [90]. These statements indicate that the increased C, N, P, and S contents of the SSC-treated soils resulted in a harmonious plant nutrient supply in the treatments, mainly in the cases of the 18 and 27 t ha⁻¹ doses.

The PCA results (Figure 2) prove that the SSC used is a very good source of plant-available P. This is very important for farmers because of the depletion of P sources and the

increasing prices of P chemical fertilizers. The limited phosphorous and nitrogen availability will reduce carbon storage in the future [19], indicating the importance of the P ratio in the carbon cycle. Moreover, using SSC as organic fertilizer contributes to a significant increase in the total N content of soil (Table 2). The values can be interpreted as follows: the low C:N ratio presumably indicates more active mineralization processes compared to immobilization if we consider the C:N ratio as a basic measure of soil decomposition. Hence, C and N are bound together in organic matter; the greater the amount of nitrogen, the greater the accumulation of organically bound carbon. Data from the literature consider the 11:1 ratio to be an optimal C:N ratio depending on land use, agronomic management, environmental conditions, the type and content of soil organic matter, and vegetation [91]. The calculated C:N and N:P ratios in our experiment were in the ranges given by the work [6]. The humus fraction of soil has constant elemental ratios; the ratio of C and P is more variable than that of C and N or C and S [13]. In our case, the E4/E6 ratios revealed that SSC treatments resulted in an increase in less complex and stable fulvic acids in the soil, which could explain the changes in C:N, C:P, and C:S ratios in the treated soils.

In the soil pH range in our experiment (4.38–6.16), the pH had the strongest effect on the measured enzyme activities, based on the CCA results (Figure 3). Increasing the pH from 4 to 6 increases the OM mineralization by around 3 times [61], which is consistent with the increase in microbial activity at higher pH levels in the treated plots.

The activity of ALP is significantly influenced not only by the total N content of the soil but also by the N:P stoichiometry of the subalpine forest soil; genes encoding ALP are under starvation response-induced transcription [92]. The role of N:P and C:P stoichiometry has already been proven in the case of ACP enzyme activity [39,82]. Our results confirm the role of the N:P ratio in ACP activity, but ALP was mainly affected by the C:N ratio (Figure 3), which is an indicator of soil organic matter decomposition, quality, and stability and the mineralization of N [93]. Organic or inorganic soil amendments affect C:N:P stoichiometry primarily through the soil microbial diversity and the trophic strategy of microorganisms [94]. Our results prove that C:P and N:P ratios were the predictive factors of changes in phosphatase enzyme activity. The increasing activity of ALP with the treatments could be a response to inorganic P availability [95].

4.4. Yield of Rye

Although the earlier development and physiological values of rye in SSC treatments were statistically better than in the control plants [85], the yield was not increased significantly (Table 6). Besides the nutrient supply, the climatic conditions from autumn to spring, the spring precipitation, and the soil physical conditions were the main factors in the yield of rye [96].

5. Conclusions

In this study, the results of 20 years of regular sewage sludge compost (SSC) application were presented. The applied SSC is a complex material of organic and inorganic constituents, designed for acidic sandy soil. Our results proved that its application can stabilize the organic matter content of plowed sandy soil and increase its C, N, P, and S contents. Although the element contents increased after SSC application, the changes in the C:N, C:S, and N:S ratios were not significant. Because carbon and phosphorus are important environmental factors in soil enzyme activities, the soil phosphatase activities (ACP and ALP) were affected by the C:N, C:P, and N:P ratios. The E4/E6 ratio indicated that SSC contains more fulvic than humic acids; therefore, the more stable OM of the control plots resulted in lower β -glucosidase activity. The ANOVA and PCA results proved that the SSC used is a very good source of plant-available P; therefore, this SSC's application

could eliminate the problems of P deficiency after adding C and N, with the SSC, to soil. Moreover, the small increase in the C:N ratio indicates the appropriate availability of nitrogen for microorganisms and plants, because N is not a limiting factor, and the rate of carbon mineralization does not depend on the N content of the soil in the treated plots. Our results revealed that, along with increasing SSC doses, the role of microbes in P mobilization became more and more important, as the increasing ALP and decreasing ACP activities indicated. The soil pH had the strongest effect on enzyme activities, as β -GLUC and ALP had positive correlations with it, while ACP decreased with the increasing pH values. Based on this long-term experiment, the SSC could improve soil health, but it strongly depends on its microbial activity and community composition.

The long-term application of sewage sludge compost could result in a specially adapted microbial community in sandy soil. Studying microbial community composition and the selection of microbes for soil inoculation products could help to improve CNPS cycles in deteriorated soils in the future.

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References

- Schlesinger, W.H.; Bernhardt, E.S. *Biogeochemistry: An Analysis of Global Change*, 4th ed.; Academic Press: London, UK, 2020; pp. 433–526.
- Xue, Y.; Kang, H.; Cui, Y.; Lu, S.; Yang, H.; Zhu, J.; Fu, Z.; Yan, C.; Wang, D. Consistent Plant and Microbe Nutrient Limitation Patterns During Natural Vegetation Restoration. *Front. Plant Sci.* **2022**, *13*, 885984. [[CrossRef](#)] [[PubMed](#)]
- Sardans, J.; Janssens, I.A.; Ciais, P.; Obersteiner, M.; Peñuelas, J. Recent advances and future research in ecological stoichiometry. *Perspect. Plant Ecol. Evol. Syst.* **2021**, *50*, 125611. [[CrossRef](#)]
- Zhang, W.; Liu, W.; Xu, M.; Deng, J.; Han, X.; Yang, G.; Feng, Y.; Ren, G. Response of forest growth to C:N:P stoichiometry in plants and soils during *Robinia pseudoacacia* afforestation on the Loess Plateau, China. *Geoderma* **2019**, *337*, 280–289. [[CrossRef](#)]
- Mooshammer, M.; Wanek, W.; Zechmeister-Boltenstern, S.; Richter, A.A. Stoichiometric imbalances between terrestrial decomposer communities and their resources: Mechanisms and implications of microbial adaptations to their resources. *Front. Microbiol.* **2014**, *5*, 22. [[CrossRef](#)] [[PubMed](#)]
- Cleveland, C.C.; Liptzin, D. C:N:P stoichiometry in soil: Is there a “Redfield ratio” for the microbial biomass? *Biogeochemistry* **2007**, *85*, 235–252. [[CrossRef](#)]
- Sinsabaugh, R.L.; Hill, B.H.; Follstad Shah, J.J. Ecoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment. *Nature* **2009**, *462*, 795–798. [[CrossRef](#)]
- Sinsabaugh, R.L.; Follstad Shah, J.J.; Hill, B.H.; Elonen, C.M. Ecoenzymatic Stoichiometry of Stream Sediments with Comparison to Terrestrial Soils. *Biogeochemistry* **2012**, *111*, 455–467. [[CrossRef](#)]
- Xu, X.; Thornton, P.; Post, W. A global analysis of soil microbial biomass carbon, nitrogen and phosphorus in terrestrial ecosystems. *Glob. Ecol. Biogeogr.* **2013**, *22*, 737–749. [[CrossRef](#)]
- Rodrigues, M.; Withers, P.J.A.; Soltangheisi, A.; Vargas, V.; Holzschuh, M.; Pavinato, P.S. Tillage systems and cover crops affecting soil phosphorus bioavailability in Brazilian Cerrado Oxisols. *Soil Till. Res.* **2021**, *205*, 104770. [[CrossRef](#)]

11. Peñuelas, J.; Janssens, I.A.; Ciais, P.; Obersteiner, M.; Sardans, J. Anthropogenic global shifts in biospheric N and P concentrations and ratios and their impacts on biodiversity, ecosystem productivity, food security, and human health. *Glob. Change Biol.* **2020**, *26*, 1962–1985. [[CrossRef](#)]
12. Xu, H.; Wang, M.; You, C.; Tan, B.; Xu, L.; Li, H.; Zhang, L.; Wang, L.; Liu, S.; Hou, G.; et al. Warming Effects on C: N: P Stoichiometry and Nutrient Limitation in Terrestrial Ecosystems. *Soil Till. Res.* **2024**, *235*, 105896. [[CrossRef](#)]
13. Kirkby, C.A.; Kirkegaard, J.A.; Richardson, A.E.; Wade, L.J.; Blanchard, C.; Batten, G. Stable soil organic matter: A comparison of C:N:P:S ratios in Australian and other world soils. *Geoderma* **2011**, *163*, 197–208. [[CrossRef](#)]
14. Amaleviciute-Volunge, K.; Tripolskaja, L.; Kazlauskaite-Jadzevice, A.; Slepeliene, A.; Baksiene, E. Effect of Long-Term Different Land Uses on Improving Stable Humic Compounds in Arenosol. *Agriculture* **2024**, *14*, 250. [[CrossRef](#)]
15. Lai, R.; Lagomarsino, A.; Ledda, L.; Roggero, P.P. Variation in soil C and microbial functions across tree canopy projection and open grassland microenvironments. *Turk. J. Agric. For.* **2014**, *38*, 62–69. [[CrossRef](#)]
16. Amorim, H.C.S.; Hurtarte, L.C.C.; Souza, I.F.; Zinn, Y.L. C: N ratios of bulk soils and particle-size fractions: Global trends and major drivers. *Geoderma* **2022**, *425*, 116026. [[CrossRef](#)]
17. Bai, T.; Wang, P.; Qiu, Y.; Zhang, Y.; Hu, S. Nitrogen availability mediates soil carbon cycling response to climate warming: A meta-analysis. *Glob. Change Biol.* **2023**, *29*, 2608–2626. [[CrossRef](#)] [[PubMed](#)]
18. Chen, L.; Liu, L.; Mao, C.; Qin, S.; Wang, J.; Liu, F.; Blagodatsky, S.; Yang, G.; Zhang, Q.; Zhang, D.; et al. Nitrogen availability regulates topsoil carbon dynamics after permafrost thaw by altering microbial metabolic efficiency. *Nat. Commun.* **2018**, *9*, 3951. [[CrossRef](#)] [[PubMed](#)]
19. Peñuelas, J.; Poulter, B.; Sardans, J.; Ciais, P.; van der Velde, M.; Bopp, L.; Olivier, B.; Godderis, Y.; Hinsinger, P.; Llusia, J.; et al. Human-induced nitrogen-phosphorus imbalances alter natural and managed ecosystems across the globe. *Nat. Commun.* **2013**, *4*, 2934. [[CrossRef](#)]
20. Siwik-Ziomek, A.; Lemanowicz, J. The content of carbon, nitrogen, phosphorus and sulphur in soil against the activity of selected hydrolases as affected by crop rotation and fertilization. *Zemdirbyste* **2014**, *101*, 367–372. [[CrossRef](#)]
21. Wang, L.; Lu, P.; Feng, S.; Hamel, C.; Sun, D.; Siddique, K.H.M.; Gan, G.Y. Strategies to improve soil health by optimizing the plant-soil-microbe-anthropogenic activity nexus. *Agr. Ecosyst. Environ.* **2024**, *359*, 108750. [[CrossRef](#)]
22. Chen, G.; Yuan, J.; Wang, S.; Liang, Y.; Wang, D.; Zhu, Y.; Wang, Y. Soil and microbial C:N:P stoichiometries play vital roles in regulating P transformation in agricultural ecosystems: A review. *Pedosphere* **2024**, *34*, 44–51. [[CrossRef](#)]
23. Bennett, J.; Klironomos, J. Mechanisms of plant–soil feedback: Interactions among biotic and abiotic drivers. *New Phytol.* **2019**, *222*, 91–96. [[CrossRef](#)] [[PubMed](#)]
24. Frene, J.P.; Pandey, B.K.; Castrillo, G. Under pressure: Elucidating soil compaction and its effect on soil functions. *Plant Soil* **2024**, *502*, 267–278. [[CrossRef](#)]
25. Norton, J.; Ouyang, Y. Controls and Adaptive Management of Nitrification in Agricultural Soils. *Front. Microbiol.* **2019**, *10*, 1931. [[CrossRef](#)] [[PubMed](#)]
26. Zavarzina, A.G.; Danchenko, N.N.; Demin, V.V.; Artemyeva, Z.S.; Kogut, B.M. Humic Substances: Hypotheses and Reality (a Review). *Eurasian Soil Sc.* **2021**, *54*, 1826–1854. [[CrossRef](#)]
27. Hartman, W.H.; Richardson, C.J. Differential nutrient limitation of soil microbial biomass and metabolic quotients (qCO_2): Is there a biological stoichiometry of soil microbes? *PLoS ONE* **2013**, *8*, e57127. [[CrossRef](#)]
28. Daunoras, J.; Kačergius, A.; Gudiukaitė, R. Role of Soil Microbiota Enzymes in Soil Health and Activity Changes Depending on Climate Change and the Type of Soil Ecosystem. *Biology* **2024**, *13*, 85. [[CrossRef](#)] [[PubMed](#)]
29. de Almeida, R.F.; Naves, E.R.; da Mota, R.P. Soil Quality: Enzymatic Activity of Soil β -Glucosidase. *Glob. J. Agric. Res. Rev.* **2015**, *3*, 146–450.
30. Sandhu, S.; Sekaran, U.; Ozlu, E.; Hoilett, N.O.; Kumar, S. Short-Term Impacts of Biochar and Manure Application on Soil Labile Carbon Fractions, Enzyme Activity, and Microbial Community Structure. *Biochar* **2019**, *1*, 271–282. [[CrossRef](#)]
31. Stefanoni Rubio, P.J.; Godoy, M.S.; Della Mónica, I.F.; Pettinari, M.J.; Godeas, A.M.; Scervino, J.M. Carbon and nitrogen sources influence tricalcium phosphate solubilization and extracellular phosphatase activity by *Talaromyces flavus*. *Curr. Microbiol.* **2016**, *72*, 41–47. [[CrossRef](#)] [[PubMed](#)]
32. Ye, J.; Zhang, R.; Nielsen, S.; Joseph, S.D.; Huang, D.; Thomas, T. A Combination of Biochar–Mineral Complexes and Compost Improves Soil Bacterial Processes, Soil Quality, and Plant Properties. *Front. Microbiol.* **2016**, *7*, 372. [[CrossRef](#)] [[PubMed](#)]
33. Dick, C.F.; Dos-Santos, A.L.; Meyer-Fernandes, J.R. Inorganic phosphate as an important regulator of phosphatases. *Enzyme Res.* **2011**, 103980. [[CrossRef](#)]
34. Bajouco, R.; Fraga, I.; Pinheiro, J.; Coutinho, J. Acid Phosphomonoesterase and β -Glucosidase Activities in Volcanic Soils Under Permanent Fertilized Pastures: Distribution Profile and Microbial Effort Toward P Acquisition. *Soil Sci. Plant Nutr.* **2020**, *66*, 734–744. [[CrossRef](#)]
35. Gou, X.; Ren, Y.; Qin, X.; Wei, X.; Wang, J. Global patterns of soil phosphatase responses to nitrogen and phosphorus fertilization. *Pedosphere* **2024**, *34*, 200–210. [[CrossRef](#)]

36. Gou, X.M.; Cai, Y.; Wang, C.Q.; Li, B.; Zhang, R.P.; Zhang, Y.; Tang, X.Y.; Chen, Q.; Shen, J.; Deng, J.R.; et al. Effects of different long-term cropping systems on *phoD*-harboring bacterial community in red soils. *J. Soils Sediments* **2021**, *21*, 376–387. [CrossRef]
37. Ibrahim, M.; Iqbal, M.; Tang, Y.-T.; Khan, S.; Guan, D.-X.; Li, G. Phosphorus Mobilization in Plant–Soil Environments and Inspired Strategies for Managing Phosphorus: A Review. *Agronomy* **2022**, *12*, 2539. [CrossRef]
38. Fujita, K.; Kunito, T.; Moro, H.; Toda, H.; Otsuka, S.; Nagaoka, K. Microbial resource allocation for phosphatase synthesis reflects the availability of inorganic phosphorus across various soils. *Biogeochemistry* **2017**, *136*, 325–339. Available online: <https://www.jstor.org/stable/48720871> (accessed on 6 January 2025). [CrossRef]
39. Margalef, O.; Sardans, J.; Fernández-Martínez, M.; Molowny-Horas, R.; Janssens, I.A.; Ciais, P.; Goll, D.; Richter, A.; Obersteiner, M.; Asensio, D.; et al. Global patterns of phosphatase activity in natural soils. *Sci. Rep.* **2017**, *7*, 1337. [CrossRef]
40. Kosobucki, P. Study of Compost Based on Sewage Sludge and Different Structural Materials. *Biomass* **2024**, *4*, 273–285. [CrossRef]
41. Myszura-Dymek, M. Properties of Organic Matter in Composts Based on Sewage Sludge. *J. Ecol. Eng.* **2024**, *25*, 70–81. [CrossRef]
42. Farsang, A.; Babcsányi, I.; Ladányi, Z.; Perei, K.; Bodor, A.; Csányi, T.K.; Barta, K. Evaluating the effects of sewage sludge compost applications on the microbial activity, the nutrient and heavy metal content of a Chernozem soil in a field survey. *Arab. J. Geosci.* **2020**, *13*, 982. [CrossRef]
43. Myszura-Dymek, M.; Zukowska, G. The Influence of Sewage Sludge Composts on the Enzymatic Activity of Reclaimed Post-Mining Soil. *Sustainability* **2023**, *15*, 4749. [CrossRef]
44. Sciubba, L.; Cavani, L.; Marzadori, C.; Ciavatta, C. Effect of biosolids from municipal sewage sludge composted with rice husk on soil functionality. *Biol. Fertil. Soils* **2013**, *49*, 597–608. [CrossRef]
45. Curci, M.; Lavecchia, A.; Cucci, G.; Lacolla, G.; De Corato, U.; Crecchio, C. Short-Term Effects of Sewage Sludge Compost Amendment on Semiarid Soil. *Soil Syst.* **2020**, *4*, 48. [CrossRef]
46. Liu, X.; Guo, K.; Huang, L.; Ji, Z.; Jiang, H.; Li, H.; Zhang, J. Responses of absolute and specific enzyme activity to consecutive application of composted sewage sludge in a Fluventic Ustochrept. *PLoS ONE* **2017**, *12*, e0177796. [CrossRef]
47. IUSS Working Group WRB. *World Reference Base for Soil Resources. International Soil Classification System for Naming Soils and Creating Legends for Soil Maps*, 4th ed.; International Union of Soil Sciences (IUSS): Vienna, Austria, 2022.
48. Buzás, I. *Manual of Soil and Agrochemical Analysis. 2. Physico-Chemical and Chemical Analytical Methods for Soils*; Mezőgazdasági Kiadó: Budapest, Hungary, 1988. (In Hungarian)
49. Hungarian Standard. (MSZ) 20135 Determination of available nutrient content of soils. In *Item 4.2.1: Preparing the Ammonium Lactate Extractant. Item 5.4.2: Determination of Orto-Phosphate Concentration*; Hungarian Standard Association: Budapest, Hungary, 1999. (In Hungarian)
50. Mulvaney, R.L. Nitrogen-inorganic forms. In *Methods of Soil Analysis, Part 3, Chemical Methods*, 1st ed.; Sparks, D.L., Page, A.L., Helmke, P.A., Loeppert, R.H., Soltanpour, P.N., Tabatabai, M.A., Johnston, C.T., Sumner, M.E., Eds.; Soil Science Society of America Inc., American Society of Agronomy, Inc.: Madison, WI, USA, 1996; SSSA Book Series No. 5; pp. 1123–1184.
51. Wuenscher, R.; Unterfrauner, H.; Peticzka, R.; Zehetner, F. A comparison of 14 soil phosphorus extraction methods applied to 50 agricultural soils from Central Europe. *Plant Soil Environ.* **2015**, *61*, 86–96. [CrossRef]
52. Page, A.L.; Miller, R.H.; Keeney, D.R. *Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties*, 2nd ed.; Agronomy Monograph 9; ASA and SSSA: Madison, WI, USA, 1982; pp. 591–592.
53. Jimenez, R.R.; Ladha, J.K. Automated elemental analysis: A rapid and reliable but expensive measurement of total carbon and nitrogen in plant and soil samples. *Commun. Soil Sci. Plan.* **1993**, *24*, 1897–1924. [CrossRef]
54. Eivazi, F.; Tabatabai, M.A. Glucosidases and galactosidases in soils. *Soil Biol. Biochem.* **1988**, *20*, 601–606. [CrossRef]
55. Eivazi, F.; Tabatabai, M.A. Phosphatases in soils. *Soil Biol. Biochem.* **1976**, *9*, 167–172. [CrossRef]
56. Hartemink, A.E.; Barrow, N.J. Soil pH—Nutrient relationships: The diagram. *Plant Soil* **2023**, *486*, 209–215. [CrossRef]
57. Puissant, J.; Jones, B.; Goodall, T.; Mang, D.; Blaud, A.; Gweon, H.S.; Malik, A.; Jones, D.L.; Clark, I.M.; Hirsch, P.R.; et al. The pH optimum of soil exoenzymes adapt to long term changes in soil pH. *Soil Biol. Biochem.* **2019**, *138*, 107601. [CrossRef]
58. Wang, C.; Zhou, X.; Guo, D.; Zhao, J.; Yan, L.; Feng, G.; Gao, O.; Yu, H.; Zhao, L. Soil pH is the primary factor driving the distribution and function of microorganisms in farmland soils in northeastern China. *Ann. Microbiol.* **2019**, *69*, 1461–1473. [CrossRef]
59. Shi, Y.; Li, Y.; Yang, T.; Chu, H. Threshold effects of soil pH on microbial co-occurrence structure in acidic and alkaline arable lands. *Sci. Total Environ.* **2021**, *800*, 149592. [CrossRef] [PubMed]
60. Penn, C.J.; Camberato, J.J. A Critical Review on Soil Chemical Processes that Control How Soil pH Affects Phosphorus Availability to Plants. *Agriculture* **2019**, *9*, 120. [CrossRef]
61. Barrow, N.J.; Hartemink, A.E. The effects of pH on nutrient availability depend on both soils and plants. *Plant Soil* **2023**, *487*, 21–37. [CrossRef]
62. Chaplot, V.; Smith, P. Cropping leads to loss of soil organic matter: How can we prevent it? *Pedosphere* **2023**, *33*, 8–10. [CrossRef]
63. Oktaba, L.; Odrobińska, D.; Uzarowicz, Ł. The impact of different land uses in urban area on humus quality. *J. Soils Sediments* **2018**, *18*, 2823–2832. [CrossRef]

64. Yang, S.; Zhang, Z.; Cong, L.; Wang, X.; Shi, S. Effect of fulvic acid on the phosphorus availability in acid soil. *J. Soil Sci. Plant Nut.* **2013**, *13*, 526–533. [\[CrossRef\]](#)
65. Zalba, P.; Amiotti, N.M.; Juan, A.; Galantini, J.A.; Pistola, S. Soil Humic and Fulvic Acids from Different Land-Use Systems Evaluated By E4/E6 Ratios. *Commun. Soil Sci. Plant Anal.* **2016**, *47*, 1675–1679. [\[CrossRef\]](#)
66. Canellas, L.P.; Olivares, F.L. Physiological responses to humic substances as plant growth promoter. *Chem. Biol. Technol. Agric.* **2014**, *1*, 3. [\[CrossRef\]](#)
67. Remick, K.A.; Helmann, J.D. Chapter One—The elements of life: A biocentric tour of the periodic table. In *Advances in Microbial Physiology*, 1st ed.; Poole, R.K., Kelly, D.J., Eds.; Academic Press: London, UK, 2023; Volume 82, pp. 1–127. [\[CrossRef\]](#)
68. Silva, M.B.; Camargos, L.S.; Teixeira Filho, M.C.M.; Souza, L.A.; Coscione, A.R.; Lavres, J.; Abreu-Junior, C.H.; He, Z.; Zhao, F.; Jani, A.D.; et al. Residual effects of composted sewage sludge on nitrogen cycling and plant metabolism in a no-till common bean-palisade grass-soybean rotation. *Front Plant Sci.* **2023**, *14*, 1281670. [\[CrossRef\]](#) [\[PubMed\]](#)
69. Telman, W.; Dietz, K.-J. Thiol redox-regulation for efficient adjustment of sulfur metabolism in acclimation to abiotic stress. *J. Exp. Bot.* **2019**, *70*, 4223–4236. [\[CrossRef\]](#) [\[PubMed\]](#)
70. Nakai, Y.; Maruyama-Nakashita, A. Biosynthesis of Sulfur-Containing Small Biomolecules in Plants. *Int. J. Mol. Sci.* **2020**, *21*, 3470. [\[CrossRef\]](#) [\[PubMed\]](#)
71. Sharma, R.K.; Cox, M.S.; Oglesby, C.; Dhillon, J.S. Revisiting the role of sulfur in crop production: A narrative review. *J. Agric. Food Res.* **2024**, *15*, 101013. [\[CrossRef\]](#)
72. Kopriva, S.; Malagoli, M.; Takahashi, H. Sulfur nutrition: Impacts on plant development, metabolism, and stress responses. *J. Exp. Bot.* **2019**, *70*, 4069–4073. [\[CrossRef\]](#) [\[PubMed\]](#)
73. Turner, B.L.; Lambers, H.; Condron, L.M.; Cramer, M.D.; Leake, J.R.; Richardson, A.E.; Smith, S.E. Soil microbial biomass and the fate of phosphorus during long-term ecosystem development. *Plant Soil* **2013**, *367*, 225–234. [\[CrossRef\]](#)
74. Liang, C.; Schimel, J.P.; Jastrow, J.D. The importance of anabolism in microbial control over soil carbon storage. *Nat. Microbiol.* **2017**, *2*, 17105. [\[CrossRef\]](#) [\[PubMed\]](#)
75. Fierer, N.; Wood, S.S.; Mesquita, C.P.B. How Microbes Can, and Cannot, Be Used to Assess Soil Health. *Soil Biol. Biochem.* **2021**, *153*, 108111. [\[CrossRef\]](#)
76. Długosz, J.; Piotrowska-Długosz, A.; Kalisz, B. Vertical changes in P-acquiring enzyme activities and microbial biomass in Luvisols—The effect of different types of agricultural land use and soil-forming processes. *Geoderma* **2023**, *432*, 116406. [\[CrossRef\]](#)
77. Margenot, A.J.; Wade, J. Getting the Basics Right on Soil Enzyme Activities: A Comment on Sainju. (2022). *Agrosys. Geosci. Environ.* **2023**, *6*, e20405. [\[CrossRef\]](#)
78. Wei, Y.; Zhao, Y.; Xi, B.; Wei, Z.; Li, X.; Cao, Z. Changes in phosphorus fractions during organic wastes composting from different sources. *Bioresour. Technol.* **2015**, *189*, 349–356. [\[CrossRef\]](#) [\[PubMed\]](#)
79. Spohn, M.; Kuzyakov, Y. Distribution of microbial- and root-derived phosphatase activities in the rhizosphere depending on P availability and C allocation—Coupling soil zymography with ¹⁴C imaging. *Soil Biol. Biochem.* **2013**, *67*, 106–113. [\[CrossRef\]](#)
80. Tan, H.; Barret, M.; Mooij, M.J.; Rice, O.; Morrissey, J.P.; Dobson, A.; Griffiths, B.; O’Gara, F. Long-term phosphorus fertilisation increased the diversity of the total bacterial community and the phoD phosphorus mineraliser group in pasture soils. *Biol. Fertil. Soils* **2013**, *49*, 661–672. [\[CrossRef\]](#)
81. Santos-Beneit, F. The Pho regulon: A huge regulatory network in bacteria. *Front. Microbiol.* **2015**, *6*, 402. [\[CrossRef\]](#) [\[PubMed\]](#)
82. Luo, G.W.; Sun, B.; Li, L.; Liu, M.Q.; Zhu, Y.Y.; Guo, S.W.; Ling, N.; Shen, Q.R. Understanding how long-term organic amendments increase soil phosphatase activities: Insight into phoD- and phoC-harboring functional microbial populations. *Soil Biol. Biochem.* **2019**, *139*, 107632. [\[CrossRef\]](#)
83. Xu, Z.; Yu, G.; Zhang, X.; He, N.; Wang, Q.; Wang, S.; Wang, R.; Zhao, N.; Jia, Y.; Wang, C. Soil enzyme activity and stoichiometry in forest ecosystems along the North-South Transect in eastern China (NSTEC). *Soil Biol. Biochem.* **2017**, *104*, 152–163. [\[CrossRef\]](#)
84. Camenzind, T.; Hättenschwiler, S.; Treseder, K.; Lehmann, A.; Rillig, M.C. Nutrient limitation of soil microbial processes in tropical forests. *Ecol. Monogr.* **2018**, *88*, 4–21. [\[CrossRef\]](#)
85. Almási, C.; Orosz, V.; Tóth, T.; Henzsel, I.; Demeter, I.; Mansour, M.M.; Makádi, M. Sewage sludge compost as an alternative source of phosphorus to rye in acidic sandy soil. *Acta Agr. Debreceniensis* **2024**, *1*, 11–18. [\[CrossRef\]](#)
86. Zuccarini, P.; Asensio, D.; Sardans, J.; Ogaya, R.; Peñuelas, J. Changes in soil enzymatic activity in a P-limited Mediterranean shrubland subject to experimental nitrogen deposition. *Appl. Soil Ecol.* **2021**, *168*, 104159. [\[CrossRef\]](#)
87. Yang, K.; Zhu, J.; Gu, J.; Yu, L.; Wang, Z. Changes in soil phosphorus fractions after 9 years of continuous nitrogen addition in a *Larix gmelinii* plantation. *Ann. For. Sci.* **2015**, *72*, 435–442. [\[CrossRef\]](#)
88. Zhou, L.; Liu, S.; Shen, H.; Zhao, M.; Xu, L.; Xing, A.; Fang, J. Soil extracellular enzyme activity and stoichiometry in China’s forests. *Funct. Ecol.* **2020**, *34*, 1461–1471. [\[CrossRef\]](#)
89. Ladau, J.; Eloe-Fadrosh, E.A. Spatial, Temporal, and Phylogenetic Scales of Microbial Ecology. *Trends Microbiol.* **2019**, *8*, 662–669. [\[CrossRef\]](#)

90. Jobe, T.O.; Zenzen, I.; Karvansara, P.R.; Kopriva, S. Integration of sulfate assimilation with carbon and nitrogen metabolism in transition from C₃ to C₄ photosynthesis. *J. Exp. Bot.* **2019**, *70*, 4211–4221. [[CrossRef](#)]
91. Torres Bazarro, J.; Sanchez, J.D.; Cayón Salinas, D.G. Nutrient accumulation models in the banana (*Musa AAA Simmonds cv. Williams*) plant under nitrogen doses. *Acta Agron.* **2017**, *66*, 391–396. [[CrossRef](#)]
92. Li, J.; Xie, T.; Zhu, H.; Zhou, J.; Li, C.; Xiong, W.; Xu, L.; Wu, Y.; He, Z.; Li, X. Alkaline phosphatase activity mediates soil organic phosphorus mineralization in a subalpine forest ecosystem. *Geoderma* **2021**, *404*, 115376. [[CrossRef](#)]
93. Amorim, H.C.S.; Araujo, M.A.; Lal, R.; Zinn, Y.L. What C: N ratios in soil particle-size fractions really say: N is preferentially sorbed by clays over organic C. *Catena* **2023**, *230*, 107230. [[CrossRef](#)]
94. Delgado-Baquerizo, M.; Reich, P.B.; Khachane, A.N.; Campbell, C.D.; Thomas, N.; Freitag, T.E.; Abu Al-Soud, W.; Sørensen, S.; Bardgett, R.D.; Singh, B.K. It is elemental: Soil nutrient stoichiometry drives bacterial diversity. *Environ. Microbiol.* **2017**, *19*, 1176–1188. [[CrossRef](#)]
95. Park, Y.; Solhtalab, M.; Thongsomboon, W.; Aristilde, L. Strategies of organic phosphorus recycling by soil bacteria: Acquisition, metabolism, and regulation. *Environ. Microbiol. Rep.* **2022**, *14*, 3–24. [[CrossRef](#)]
96. Kim, M.; Peng, J.; Sung, K. Causality of climate and soil factors affecting whole crop rye (*Secale cereale* L.) yield as part of natural ecosystem structure via longitudinal structural equation model in the Republic of Korea. *Grassl. Sci.* **2020**, *66*, 110–115. [[CrossRef](#)]

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