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Foliar applications of a zeolite-based biostimulant affect soil enzyme activity and N uptake in maize and wheat under different levels of nitrogen fertilization

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ABSTRACT

There is a growing interest in developing agricultural practices that can improve crop performance while preserving natural resources. Plant biostimulants are thought to play a role in reaching this goal, in particular by increasing the nitrogen use efficiency. However, a notable research gap exists concerning the effects of foliar applications of natural zeolites as plant biostimulants on crop performance. To address this knowledge gap, a greenhouse experiment was set up in order to study the response of maize and wheat traits, specifically the biomass productivity and nitrogen uptake, as well as the response of soil extracellular enzymes to the foliar applications of a natural zeolite in combination with different levels of nitrogen fertilization, that is, 100%, 75%, and 50% of the optimal dose. Foliar application of zeolite in wheat and maize plants led to an increase in root nitrogen concentration of about 10%, particularly at the lowest nitrogen fertilization rate. This response was accompanied by an increase in aboveground to belowground uptake nitrogen ratio. Furthermore, there was a significant reduction of about 20% in root biomass in both crops with zeolite application across the entire nitrogen fertilization gradient. These plant-level responses were associated with a significant increase in the activity of carbon-degrading and nitrogen-degrading enzymes at the soil level in response to zeolite applications. Our findings provide a compelling proof-of-concept for the beneficial effects of foliar-applied zeolite as a biostimulant for crops, emphasizing the critical need for additional field research to validate our greenhouse results.

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Introduction

Meeting the demands of a rapidly expanding world population and minimizing the environmental consequences of agronomic practices pose significant challenges for modern agriculture (Clark and Tilman 2017; Lynch et al. 2021). Plant biostimulants (PBs) have been identified as important components in promoting sustainable agriculture (Cataldo et al. 2021). Indeed, PBs are defined as substances, mixtures or microorganisms that are capable of enhancing nutrient use efficiency, abiotic stress tolerance and/or crop traits (Rouphael and Colla 2020; Del Buono 2021; Du Jardin 2015) and can be classified into six categories, that is, humic and fulvic acids, protein

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hydrolysates, seaweed and other plant extracts, chitosan, beneficial fungi and bacteria, as well as inorganic compounds (Du Jardin 2015; Yakhin et al. 2016).

While the mode of actions of different PBs are yet to be fully understood (Yakhin et al. 2017), recent application of plant phenomics combined with metabolomics seems promising to elucidate how different PBs can promote plant growth (Nephali et al. 2020; Li et al. 2022; Zhang et al. 2023). PBs action should translate into economic and environmental advantages such as higher crop yield, increased crop quality, reduced fertilization rate, and/or higher stress tolerance. Recent meta-analyses have shown that PBs can enhance crop yield (Li, van Gerrewey, and Geelen 2022) and nutrient use efficiency (Herrmann et al. 2022) depending on type of biostimulant, application mode, crop species and environmental conditions.

The PBs category of inorganic compounds includes essential elements or inorganic salts such as, for example, silicates, phosphites, and carbonates. Siliceous natural nanomaterials such as zeolites can potentially be included in this category of inorganic PBs (Constantinescu-Aruxandei et al. 2020; Mondal et al. 2021). Natural zeolites are nanoporous, crystalline, hydrated aluminosilicates of alkali and alkaline earth cations that are characterized by reversible dehydration, large volume of free space, and high cation exchange capacity (Mumpton 1999). When applied as soil amendment, recent studies have shown that natural zeolites can affect soil microbial structure (Ferretti et al. 2018), reduce nitrogen losses (Ruser and Schulz 2015; Faccini et al. 2018; Akbari et al. 2021; Roumani and Olf 2021) and increase plant nutrient use efficiency (de Campos Bernardi et al. 2016; Mehrab et al. 2016; Medoro et al. 2022). Interestingly, zeolite addition to agricultural soils has been linked to changes in extracellular enzyme activities so suggesting that zeolite can also affect microbial activity and, ultimately, nutrient cycling (Garau et al. 2007; Gondek et al. 2023; Khati et al. 2017; Tzanakakis et al. 2021). However, studies that test natural zeolites as PBs for plant nutrition *via* foliar application are limited (e.g. El-Gabieri and Ata Allah 2017; Moale et al. 2021; Petoumenou 2023; Sirbu et al. 2023) and, to our knowledge, no study has yet examined the effects of zeolite on crop nutrition (but see Sedaghat et al. 2022 as a strategy against drought in wheat plants).

In order to understand the potentials of zeolites as biostimulant for crops, a greenhouse experiment has been performed where maize and spring wheat plants were treated with a natural zeolite *via* foliar application in combination with three levels of nitrogen (N) fertilization, that is, 100%, 75% and 50% of the recommended optimal dose. The tested zeolite as biostimulant (Fertiroc®) is a combination of natural zeolite (chabazite) and natural soft calcium carbonate. Our study aims to show the potential of zeolites as biostimulants in crop production by improving nutrient use and soil enzyme activity. Specifically, we want to answer the following questions: (1) how does foliar-applied zeolite affect aboveground and belowground biomass as well as the corresponding N uptake in maize and wheat plants? (2) Does soil activity of carbon, phosphorus, and N-degrading enzymes respond to foliar application of zeolite? The overarching hypothesis of this study is that, under reduced levels of N fertilization, the application of zeolite can not only improve the N nutrition of the two crops, but also stimulate the activity of soil N-degrading enzymes.

Material and methods

Experimental setup

A greenhouse experiment was conducted at the research station of the Swiss Institute for Agricultural Research (Agroscope) in Changins, Switzerland (46°24' N, 06°14' E), from April to June 2021. Spring wheat (*Triticum aestivum* L. cv. Diavel) and maize (*Zea mays* L. cv. LG31211) plants were grown in two distinct greenhouse modules under the same conditions, that is, a light/dark regime of 14/10 h with supplemental lighting of 400 W m⁻² at 22/15 °C and relative

humidity of 50%. Wheat and maize plants were grown in plastic pots of, respectively, 20 cm (diameter) \times 15 cm (height) and 20 cm (diameter) \times 25 cm (height) containing 3 kg of soil for the wheat and 6 kg of soil for the maize plants. All pots within each greenhouse module were placed on tables and randomly reshuffled every week to avoid any potential effects of microclimatic gradients.

Five and three seeds of, respectively, wheat and maize were sown in each pot. After seed emergence, maize plants were thinned to one plant per pot whereas five plants of wheat were left in each pot. All the pots contained a loamy (338 g kg⁻¹ sand, 425 g kg⁻¹ silt and 237 g kg⁻¹ clay) soil that was manually collected from the surface layer (0–15 cm) of an agricultural soil classified as Calcaric Cambisol and situated close to the greenhouse. Before potting, the soil was air-dried, ground, sieved through a 1-cm mesh and mixed thoroughly. The initial soil physico-chemical properties were as follow: pH (1:2.5, H₂O) of 7.9, total N content of 1.21 g kg⁻¹, total organic C content of 13.3 g kg⁻¹, available P content of 0.14 g kg⁻¹, and available K content 0.29 g kg⁻¹. All plants received deionized water two or three times per week so to keep soil moisture content at 60–80% of field capacity throughout the experimental period. Both crop species were allowed to grow for nine weeks. All pots received a basal fertilization treatment consisting of P (as KH₂PO₄) and K (as KCl).

Nitrogen and zeolite treatments

Pots were arranged in a replicated and completely randomized design. Nitrogen fertilization was applied at three different rates (= treatments), that is, the amount of 110 and 120 kg N ha⁻¹ for maize and wheat, respectively, in accordance with the official recommendations for optimal N fertilization (Sinaj and Richner 2017), and two lower amounts of 60 kg N ha⁻¹ and 90 kg N ha⁻¹ for both crops. Nitrogen was applied as ammonium nitrate in two dates for maize, that is, at V4 (4 leaf collars present) and V6 (six leaf collars present) growth stage, and in three dates for wheat, that is, at Zadok's growth scale of 16 (six unfolded leaves), 18 (eight unfolded leaves) and 19 (nine unfolded leaves) (Zadoks et al. 1974). In the case of maize, each of the two dates of N fertilization provided half of the total optimal N dose. Similarly, in the case of wheat fertilization, each of the three dates of N addition provided one-third of the total optimal N dose. The three N fertilization treatments (hereafter N120/N110, N60, and N90 kg ha⁻¹) were coupled with (+Z) or without (0Z) foliar application of zeolite. Thereby, the overall experiment design resulted in six treatments, that is, 3 N levels \times 2 zeolite levels. More specifically, the maize experiment consisted of 24 experimental units (each pot containing one plant was one experimental unit). For each N treatment (i.e. 110, 90 and 60 kg ha⁻¹), maize plants received two levels of foliar zeolite (addition and no-addition, see below for the specific amount) with four replicates for each treatment. For the wheat experiment, each experimental unit was represented by a pot containing five plants. The N levels and the zeolite levels followed the same protocol as for the maize so yielding to 24 experimental units. All the treatments with the foliar zeolite were sprayed on the same days when the N fertilization was applied (30, 36, and 42 days after sowing for the wheat, and 36 and 51 days after sowing for the maize). The commercial product Fertiroc® was used as natural zeolite and was sprayed over the plants with a pressurized portable sprayer of 1 L. The Fertiroc® product is a composition of natural zeolite (chabazite) mixed with a proportion of natural soft calcium carbonate. To be effective, the composition is micronized and works extremely fine according to the know-how of the producer company Power the Nature SA (Lausanne, Switzerland and Mondorf-les-Bains, Luxembourg). For wheat, the proper amount of zeolite was prepared based on the surface that was covered by 12 pots (i.e. 1.2 m²) following the manufacturer's recommendations of 10 L of Fertiroc® ha⁻¹ in 600 l of water. This resulted in the application of a solution of 72 mL of water containing 1.2 mL of Fertiroc®. For the maize, the proper amount of zeolite for 24 pots was prepared with the equivalent of 15 L of Fertiroc® ha⁻¹ in 600 L resulting in a solution of

72 ml of water containing 1.8 mL of Fertiroc[®]. The controls of the zeolite treatment (0Z) were sprayed with the same amount of deionized water on the same dates of foliar applications of Fertiroc[®].

Sampling and laboratory analyses of plant and soil samples

Wheat and maize plants were sampled after 9 weeks from the sowing in order to recover the aboveground and belowground biomass (roots). The wheat plants were sampled at the dough development stages (growth stage 85), that is, when spikes were already emerged and the shoots, including the flag leaves, were yellow (Zadoks et al. 1974). On the other hand, maize plants were sampled at the tasseling stage. For wheat, all the five plants in each pot were sampled together and then separated into roots, aboveground biomass and spike biomass. Maize plants, one per pot, were separated into aboveground (tassels were included) biomass and roots. Aboveground biomass was cut at the soil level and roots were gently washed by hand over a sieve using tap water to remove soil particles. All the separated parts were oven dried at 60 °C for 72 h.

After drying, the roots, the aboveground biomass and the spike biomass samples were ground with a rotor mill to a fine powder (<2 mm). Total N content (%) was determined by Dumas dry combustion. The N uptake in the aboveground as well as in the belowground biomass was calculated as: [N concentration (%) × dry weight (g plant⁻¹)]. Upon harvest, composite soil samples made of three soil cores (2.5 cm diameter × 10 cm depth) per pot were collected and thoroughly mixed. Fresh subsamples were immediately frozen at -20 °C for soil enzyme analysis.

Soil extracellular enzymatic activity

The activities of five selected soil enzymes related to C-cycling, i.e. β -glucosidase (BG), β -xylosidase (BX), to C and N-cycling, that is, β -N-acetylglucosaminidase (NAG), to N-cycling, that is, leucine aminopeptidase (LAP), and to P-cycling, that is, acid phosphatase (AP), were measured using synthetic fluorogenic substrates according to a modified procedure by (Marx et al. 2001; German et al. 2011). Fluorogenic 4-methylumbelliferone (MUF)-based substrate was used to determine the activities of BG, BX, NAG and AP (Sigma-Aldrich, St. Louis, MO). Fluorogenic 7-amino-4-methylcoumarin (MUC)-based substrate was used to determine the activity of LAP (Sigma-Aldrich, St. Louis, MO). The fluorescence was measured by a microplate reader (BioTek, Instruments, US) with excitation wavelength of 355 nm and emission wavelength of 460 nm. For calibration and quenching effects, a set of standards were prepared with 200 μ L of soil slurry solution (for each individual sample) with a range of increasing concentrations of MUF or MUC standards. Enzyme activities were calculated from the regression slopes of the standard measurements along with the fluorescence average values of the triplicates for each sample and they were reported as μ mol substrate (MUF or MUC) g⁻¹ dry soil h⁻¹.

Statistical analyses

Univariate and multivariate statistical analyses were performed using the software Statistica v. 13.5.1.17 (TIBCO Software). The experimental design had two factors, that is, N fertilization (three levels) and zeolite biostimulant (two levels). For all studied variables, a two-way analysis of variance (ANOVA) was used to test the effects of N fertilization, zeolite and their interaction. A significant interaction indicates that the effect of zeolite depends on the level of N fertilization. Within each level of N fertilization, the effect of zeolite application was tested by means of a *t*-Student test. Finally, a principal component analysis (PCA) was applied to determine the correlations and explore the variability between plant and soil parameters.

Results

Effect of N fertilization and zeolite addition on aboveground biomass

In maize plants, on the basis of two-way ANOVA, a positive effect of N fertilization on aboveground biomass and the correspondent N concentration was observed (Figure 1A, B). Instead, the zeolite addition did not have any effect either on aboveground biomass or correspondent N concentration, with the only exception of the lowest N fertilization treatment (N60) where the application of zeolite significantly increased the N concentration in aboveground biomass (+13%) compared to the no-zeolite treatment (Figure 1B).

In wheat plants, neither N fertilization nor zeolite application had a significant impact on aboveground biomass (Figure 2A). Differently, N fertilization positively affected the N

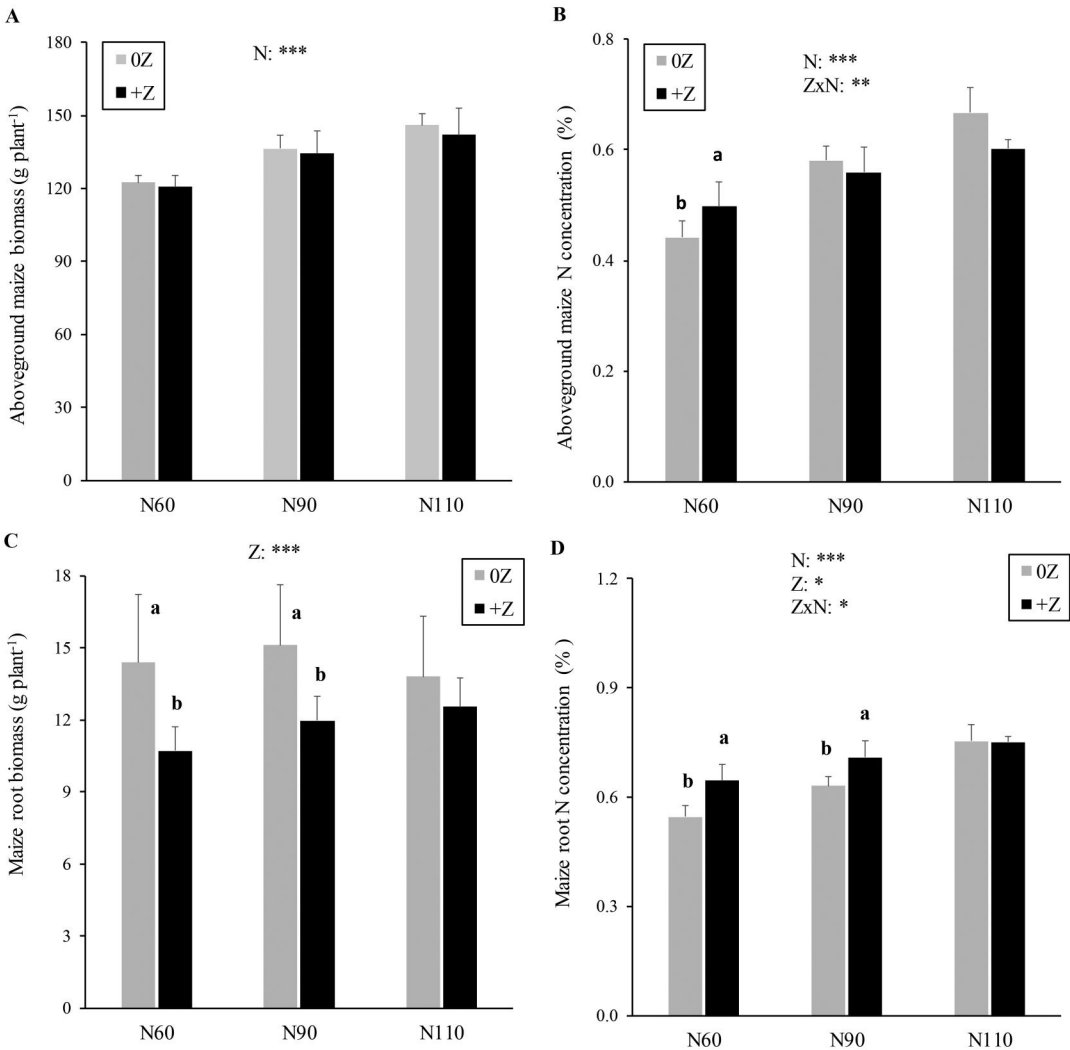


Figure 1. Mean ($n=4$) aboveground biomass (a), aboveground biomass N concentration (B), belowground biomass (C), and belowground biomass N concentration (D) in maize plants under different N (N60, N90, and N110 kg ha⁻¹) and zeolite (0Z = control, +Z = addition) treatments. Error bars represent standard deviation. The significant factors (N and Z) and their interaction (ZxN) from the two-way ANOVA are indicated (* $p < .10$, ** $p < .05$, *** $p < .01$) in relation to N and zeolite treatment. Superscript letters indicate significant differences ($p < 0.085$) between zeolite treatments for the same rate of N fertilization based on t -Student test.

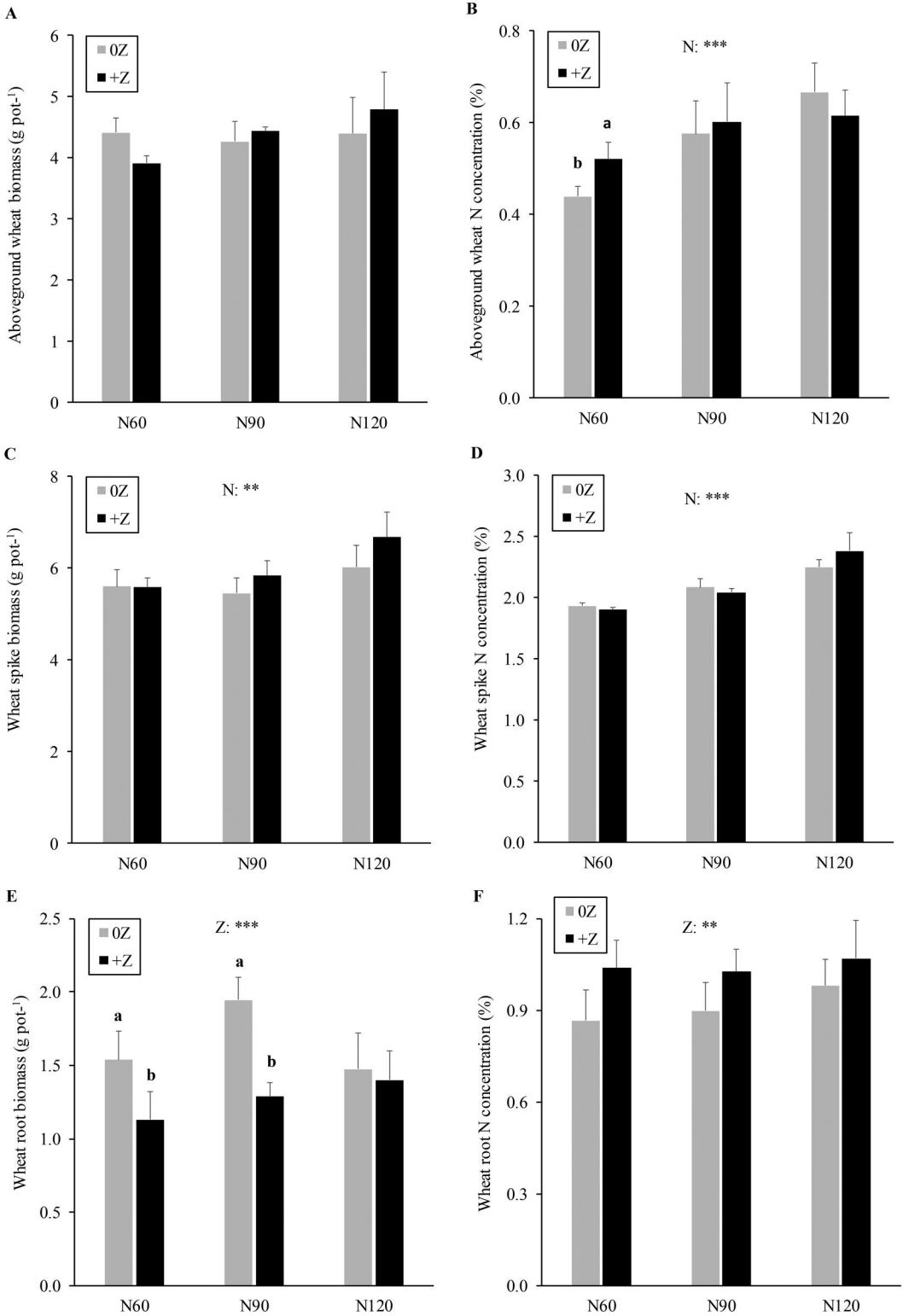


Figure 2. Mean ($n = 4$) aboveground biomass (a), aboveground biomass N concentration (B), spike biomass (C), N concentration in spike biomass (D), root biomass (E), and belowground biomass N concentration (F) in wheat plants under different nitrogen (N60, N90, and N120 kg ha⁻¹) and zeolite (0Z = control, +Z = addition) treatments. Error bars represent standard deviation. The significant factors (N and Z) and their interaction (ZxN) of the two-way ANOVA are indicated (* $p < 0.10$; ** $p < 0.05$; *** $p < 0.01$) in relation to nitrogen and zeolite treatment. Superscript letters indicate significant differences ($p < 0.040$) between zeolite treatments for the same rate of N fertilization based on t -Student test.

concentration in aboveground biomass (Figure 2B). Zeolite application did not affect the N concentration in aboveground biomass of wheat, but it did lead to a significant increase (c. +19%) in N concentration at the lowest N fertilization level compared to the no-zeolite treatment (Figure 2B). Regarding the spike biomass and N concentration in spikes (Figure 2C, D), N fertilization had a significant effect, while zeolite application did not. Nevertheless, there was a trend toward higher mean values of spike biomass with zeolite addition along the N gradient (Figure 2C).

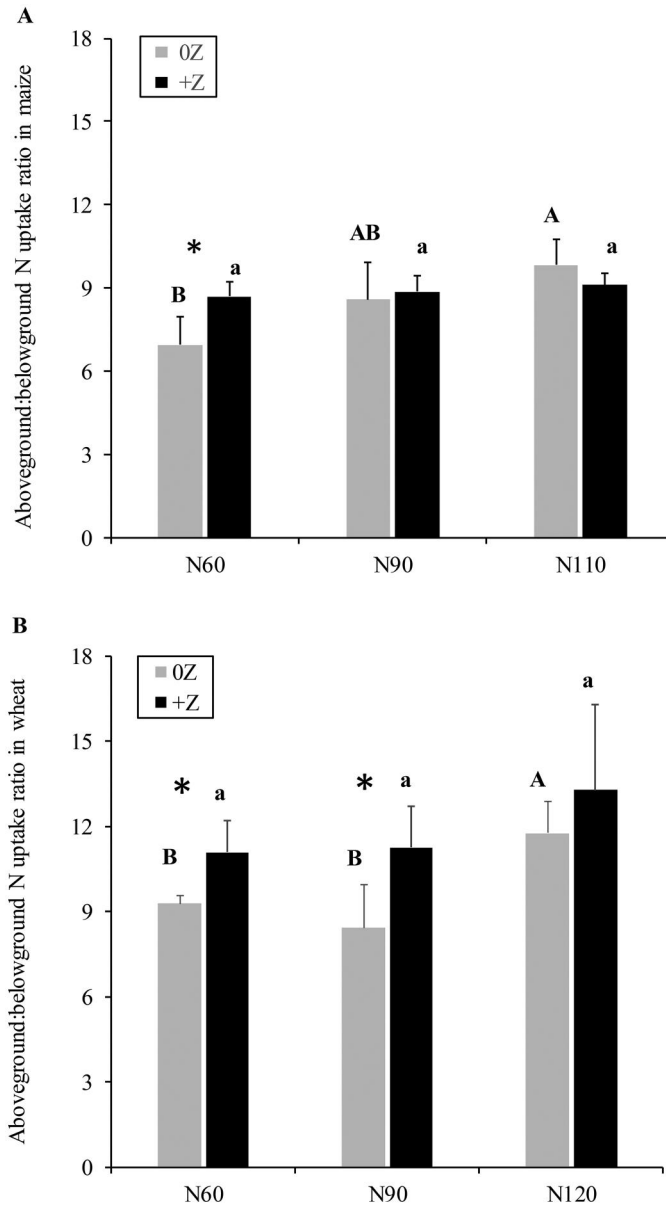


Figure 3. Mean ($n=4$) ratio of aboveground to belowground N uptake in maize plants (A) and in wheat plants (B) under different N (N60, N90, and N110/120 kg ha⁻¹) and zeolite (0Z = control, +Z = addition) treatments. In the case of wheat, the aboveground biomass includes the aerial biomass and the spike biomass. Error bars represent the standard deviation. Superscript letters indicate significant differences ($p < 0.05$) between N fertilization treatments for the same zeolite treatment (uppercase = 0Z, lowercase = +Z) based on one-way ANOVA and post-hoc Fisher LSD test. The asterisks indicate a significant difference ($p < 0.05$) between zeolite treatments for the same N fertilization level based on t -Student test.

Table 1. Effect of nitrogen (N) fertilization and zeolite (Z) addition on mean activity of C, N, and P-targeting enzymes in maize and wheat soil.

Soil	Enzyme activity ($\mu\text{mol MUF or MUC g}^{-1} \text{ soil h}^{-1}$)	Zeolite treatment		Statistical analysis		
		OZ	+Z	Z effect	N effect	ZxN interaction
Maize	Acid phosphatase (AP)	10.7 ^a	6.6 ^b	***	NS	NS
	β -glucosidase (BG)	39.4 ^b	73.3 ^a	***	NS	NS
	β -xylosidase (BX)	10.2 ^a	4.4 ^b	***	NS	NS
	N-acetylglucosaminidase (NAG)	9.3 ^a	3.9 ^b	***	NS	NS
	Leucine-aminopeptidase (LAP)	19.2 ^b	34.3 ^a	***	NS	NS
Wheat	Acid phosphatase (AP)	18.9 ^a	9.9 ^b	***	**	NS
	β -glucosidase (BG)	30.2 ^b	93.7 ^a	***	***	***
	β -xylosidase (BX)	6.9 ^b	21.3 ^a	***	NS	NS
	N-acetylglucosaminidase (NAG)	8.7 ^a	5.5 ^b	**	***	NS
	Leucine aminopeptidase (LAP)	17.1 ^b	36.8 ^a	***	NS	NS

Note: Values are the average ($n=12$) across three N fertilization rates for the two zeolite treatments. The OZ treatment indicates no-zeolite addition (control), whereas +Z indicates zeolite addition according to manufacturing indications. The statistical analysis, based on a two-way NOVA, shows the significance of zeolite and nitrogen effect as well as their interaction: NS = no-significant ($p > 0.10$), * = $p \leq 0.10$, ** = $p \leq 0.05$, *** = $p < 0.01$. For each enzyme, the significance of the Fisher-LSD post-doc comparison between zeolite treatments is indicated by different small case letters ($p \leq 0.05$).

Effect of N fertilization and zeolite addition on belowground biomass

For what concerns the belowground biomass of both crops, N fertilization did not have a significant impact on root productivity. However, there was a tendency for root biomass to decrease at higher N fertilization rates, particularly in maize (Figures 1C and 2E). Conversely, the addition of zeolite resulted in a significant decrease of root biomass for both crop species (Figures 1C and 2E) and an increase in root N concentration (Figures 1D and 2F) in all the N fertilization treatments.

Effect of zeolite addition on N uptake

The aboveground to belowground N uptake ratio was positively affected by zeolite addition for both crop species, particularly at lower N fertilization levels (Figure 3). Indeed, without zeolite application the plants receiving a higher N fertilization were characterized by a significantly higher aboveground to belowground N uptake ratio. However, with zeolite application no significant differences were observed in the N uptake ratio among the three N fertilization levels (Figure 3). Notably at the lowest N fertilization levels (N60 for maize and N60 and N90 for wheat), zeolite application resulted in a significant increase in the aboveground to belowground N uptake ratio compared to the corresponding control (Figure 3).

Effect of zeolite addition on soil enzyme activity

The foliar application of zeolite was associated with a significant change in the activity of soil enzymes in both maize and wheat soil. Specifically, the activity of β -glucosidase (BG) and leucine-aminopeptidase (LAP) increased more than double, whereas the activity of acid phosphatase (AP) and N-acetylglucosaminidase (NAG) decreased with the application of zeolite (Table 1). Furthermore, the activity of β -xylosidase (BX) increased in wheat soil, but decreased in maize soil upon zeolite application (Table 1).

Discussion

The observed higher aboveground biomass in response to N fertilization is in agreement with previous studies reporting a positive effect of increasing N availability for maize and wheat productivity (Miao et al. 2006; Morris et al. 2018; Struck et al. 2019; Ordóñez et al. 2020).

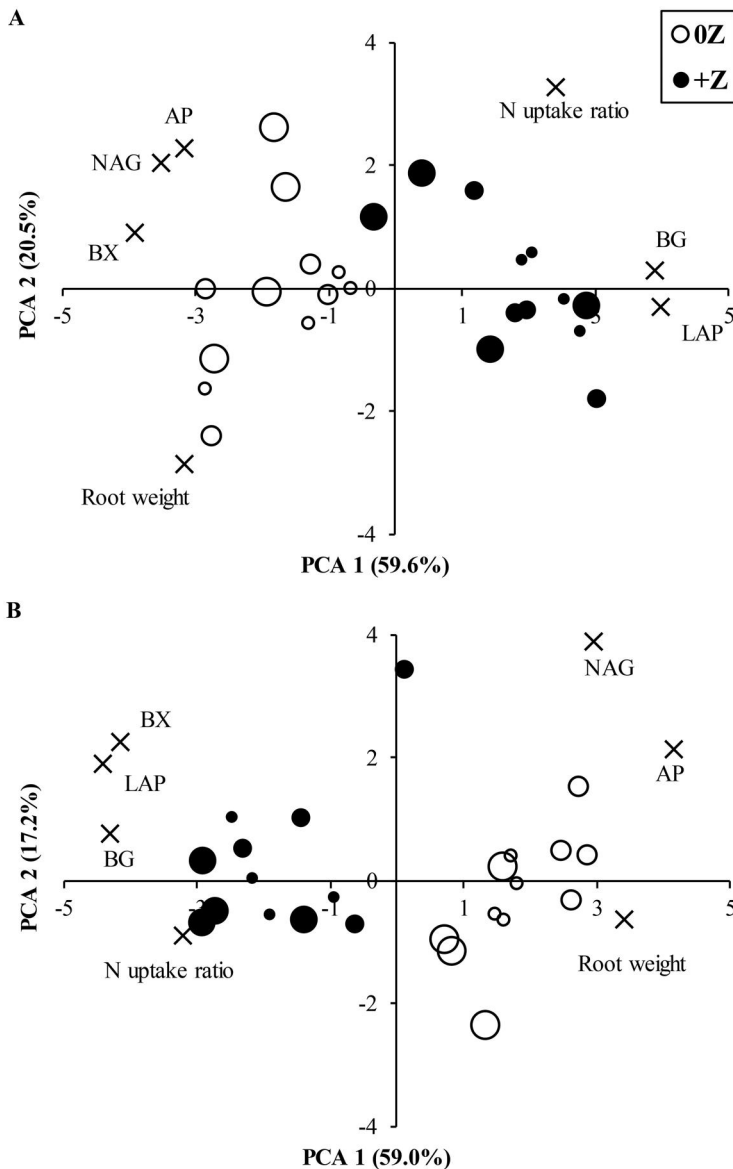


Figure 4. PCA ordination of maize (A) and wheat (B) samples on the basis of soil enzymatic activity of acid phosphatase (AP), β -glucosidase (BG), β -xylosidase (BX), N-acetylglucosaminidase (NAG), and leucine aminopeptidase (LAP) as well as on the basis of aboveground to belowground N uptake ratio and root weight under zeolite addition (+Z) and no-zeolite addition (0Z). The three N fertilization rates are represented by the increasing size of the circles (120/110 kg N ha⁻¹ = biggest size, 60 kg N ha⁻¹ = lowest size).

Interestingly, the application of zeolite in the lowest N fertilization treatment resulted in higher N concentration in aboveground biomass for both crop species, suggesting that zeolite may, directly or indirectly, enhance the N uptake under lower N availability.

Regarding the belowground productivity, previous studies have demonstrated that plants tend to allocate more dry matter to belowground biomass in order to acquire more nutrients when faced with lower N availability (Chen et al. 2015; Liu et al. 2017; Duncan et al. 2018; Ordonez et al. 2020; Kubar et al. 2022). This explains the observed increase in root biomass in response to decreasing N fertilization, a response particularly pronounced in maize. At the same time, the

observed decrease of root biomass with zeolite application may, indeed, reflect an increase of N availability, so resulting in less investment in root growth (Figures 1D and 2F). Despite the decrease in root biomass, the data indicate that zeolite application was associated with an improvement of the efficiency of N uptake, particularly under lower N fertilization where a higher N concentration in aboveground biomass was detected with zeolite application (Figures 1B and 2B).

The foliar application of zeolite led to a stabilization of the N uptake ratio across all the levels of N fertilization, particularly at low N fertilization rates (Figure 3). This effect was observed in both studied crops and was not evident in the absence of zeolite application. The direct and indirect mechanisms of zeolite for promoting a better N uptake at lower N fertilization level are still unclear and further studies are necessary. We can speculate that zeolite can either directly affect N metabolism of the plant (Li et al. 2022; Savarese et al. 2022) or indirectly through plant-soil microbial interactions (Pantigoso et al. 2022; Costa et al. 2023). For our study, we hypothesize that a role in the improvement of N uptake is also played by a change in soil enzyme activity with zeolite application (Table 1). Specifically, higher activities of both β -glucosidase (BG) and leucine-aminopeptidase (LAP) were associated to higher aboveground to belowground N ratio and lower root biomass in both crops (Figure 4). The increased LAP activity indicates an increase in amino acid degradation and, consequently, more N available to the crops (Siwik-Ziomek and Szczepanek 2019; Greenfield et al. 2021). In parallel, the increase in BG activity may reflect a change in root exudate quality and quantity so to promote soil microbial activity (Sanaullah et al. 2016) and accelerate soil N cycling (Henneron et al. 2020). The observed decrease in NAG activity is consistent with previous findings on the response of this enzyme to increased N availability (Olander and Vitousek 2000) and to changes in root exudate composition (Hao et al. 2022). The decrease of AP activity may be attributed to an increased mobilization of inorganic phosphorus resulting from the release of organic acids by plant roots (Zhang et al. 2019). Taken together, we hypothesize that the observed changes in soil enzyme activities induced by the zeolite application may reflect a functional response of the soil microbiome to changes in crop root exudates (Sieradzki et al. 2023). This link between soil enzyme activity and soil microbiome composition has been already reported in response to the addition of other types of PBs (Khatai et al. 2017; Barone et al. 2019; Mattarozzi et al. 2020; Trivedi et al. 2021).

In conclusion, this study demonstrates that foliar application of the Fertiroc® has significant effects at both plant and soil level. For both maize and wheat, zeolite addition reduced root bio-mass but increased root N concentration, especially at lower N fertilization rates. Remarkably, zeolite addition maintained a consistent aboveground to belowground N uptake ratio, even under reduced N input. At soil level, zeolite addition increased the activity of β -glucosidase and leucine-aminopeptidase enzymes potentially leading to increased N availability. This research provides a proof-of-concept for the potential benefits of foliar application of zeolite as biostimulant for crops. However, the complexity of the effects merits further in-depth investigation to fully elucidate the underlying mechanisms. This is particularly important if foliar application of zeolite can be combined with strategies aiming at reducing N fertilization levels while maintaining crop yield quality and quantity.

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Disclosure statement

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Data availability statement

The data presented in this study are available on request from the corresponding author.

References

- Akbari, H., S. A. M. Modarres-Sanavy, and A. Heidarzadeh. 2021. Fertilizer systems deployment and zeolite application on nutrients status and nitrogen use efficiency. *Journal of Plant Nutrition* 44 (2):196–212. doi: [10.1080/01904167.2020.1806299](https://doi.org/10.1080/01904167.2020.1806299).
- Barone, V., I. Puglisi, F. Fragalà, P. Stevanato, and A. Baglieri. 2019. Effect of living cells of microalgae or their extracts on soil enzyme activities. *Archives of Agronomy and Soil Science* 65 (5):712–26. doi: [10.1080/03650340.2018.1521513](https://doi.org/10.1080/03650340.2018.1521513).
- Cataldo, E., L. Salvi, F. Paoli, M. Fucile, G. Masciandaro, D. Manzi, C. M. Masini, and G. B. Mattii. 2021. Application of zeolites in agriculture and other potential uses: A review. *Agronomy* 11 (8):1547. doi: [10.3390/agronomy11081547](https://doi.org/10.3390/agronomy11081547).
- Chen, Y., J. Zhang, Q. Li, X. He, X. Su, F. Chen, L. Yuan, and G. Mi. 2015. Effects of nitrogen application on post-silking root senescence and yield of maize. *Agronomy Journal* 107 (3):835–42. doi: [10.2134/agronj14.0509](https://doi.org/10.2134/agronj14.0509).
- Clark, M., and D. Tilman. 2017. Comparative analysis of environmental impacts of agricultural production systems, agricultural input efficiency, and food choice. *Environmental Research Letters* 12 (6):064016. doi: [10.1088/1748-9326/aa6cd5](https://doi.org/10.1088/1748-9326/aa6cd5).
- Constantinescu-Aruxandei, D., C. Lupu, and F. Oancea. 2020. Siliceous natural nanomaterials as biorationals – plant protectants and plant health strengtheners. *Agronomy* 10 (11):1791. doi: [10.3390/agronomy10111791](https://doi.org/10.3390/agronomy10111791).
- Costa, O. Y. A., A. Pijl, J. Houbraken, W. van Lith, and E. E. Kuramae. 2023. Soil substrate source drives the microbes involved in the degradation of gelatin used as a biostimulant. *Applied Soil Ecology* 189:104906. doi: [10.1016/j.apsoil.2023.104906](https://doi.org/10.1016/j.apsoil.2023.104906).
- de Campos Bernardi, A. C., J. C. Polidoro, M. B. de Melo Monte, E. I. Pereira, C. R. de Oliveira, and K. Ramesh. 2016. Enhancing nutrient use efficiency using zeolites minerals - a review. *Advances in Chemical Engineering and Science* 06 (04):295–04. doi: [10.4236/aces.2016.64030](https://doi.org/10.4236/aces.2016.64030).
- Del Buono, D. 2021. Can biostimulants be used to mitigate the effect of anthropogenic climate change on agriculture? It is time to respond. *The Science of the Total Environment* 751:141763. doi: [10.1016/j.scitotenv.2020.141763](https://doi.org/10.1016/j.scitotenv.2020.141763).
- Du Jardin, P. 2015. Plant biostimulants: Definition, concept, main categories and regulation. *Scientia Horticulturae* 196:3–14. doi: [10.1016/j.scienta.2015.09.021](https://doi.org/10.1016/j.scienta.2015.09.021).
- Duncan, E. G., C. A. O’Sullivan, M. M. Roper, J. Palta, K. Whisson, and M. B. Peoples. 2018. Yield and nitrogen use efficiency of wheat increased with root length and biomass due to nitrogen, phosphorus, and potassium interactions. *Journal of Plant Nutrition and Soil Science* 181 (3):364–73. doi: [10.1002/jpln.201700376](https://doi.org/10.1002/jpln.201700376).
- El-Gabieri, A. E., and Y. F. A. Ata Allah. 2017. Effect of Foliar Application with Bentonite on Growth and Productivity of Egyptian Cotton. *Journal of Plant Production* 8 (10):1029–35. doi: [10.21608/jpp.2017.41081](https://doi.org/10.21608/jpp.2017.41081).
- Faccini, B., D. Di Giuseppe, G. Ferretti, M. Coltorti, N. Colombani, and M. Mastrociccio. 2018. Natural and NH₄⁺-enriched zeolite amendment effects on nitrate leaching from a reclaimed agricultural soil (Ferrara Province, Italy). *Nutrient Cycling in Agroecosystems* 110 (2):327–41. doi: [10.1007/s10705-017-9904-4](https://doi.org/10.1007/s10705-017-9904-4).
- Ferretti, G., K. M. Keiblinger, D. Di Giuseppe, B. Faccini, N. Colombani, S. Zechmeister-Boltenstern, M. Coltorti, and N. Mastrociccio. 2018. Short-term response of soil microbial biomass to different chabazite zeolite amendments. *Pedosphere* 28 (2):277–87. doi: [10.1016/S1002-0160\(18\)60016-5](https://doi.org/10.1016/S1002-0160(18)60016-5).
- Garau, G., P. Castaldi, L. Santona, P. Deiana, and P. Melis. 2007. Influence of red mud, zeolite and lime on heavy metal immobilization, culturable heterotrophic microbial populations and enzyme activities in a contaminated soil. *Geoderma* 142 (1-2):47–57. doi: [10.1016/j.geoderma.2007.07.011](https://doi.org/10.1016/j.geoderma.2007.07.011).

- German, D. P., M. N. Weintraub, A. S. Grandy, C. L. Lauber, Z. L. Rinkes, and S. D. Allison. 2011. Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies. *Soil Biology and Biochemistry* 43 (7):1387–97. doi: [10.1016/j.soilbio.2011.03.017](https://doi.org/10.1016/j.soilbio.2011.03.017).
- Gondek, K., M. Mierzwa-Hersztek, and R. Jarosz. 2023. Effect of willow biochar and fly ash-derived zeolite in immobilizing heavy metals and promoting enzymatic activity in a contaminated sandy soil. *Catena* 232:107429. doi: [10.1016/j.catena.2023.107429](https://doi.org/10.1016/j.catena.2023.107429).
- Greenfield, L. M., B. S. Razavi, N. Bilyera, X. Zhang, and D. L. Jones. 2021. Root hairs and protein addition to soil promote leucine aminopeptidase activity of *Hordeum vulgare* L. *Rhizosphere* 18:100329. doi: [10.1016/j.rhisph.2021.100329](https://doi.org/10.1016/j.rhisph.2021.100329).
- Hao, C., J. A. J. Dungait, X. Wei, T. Ge, Y. Kuzyakov, Z. Cui, J. Tian, and F. Zhang. 2022. Maize root exudate composition alters rhizosphere bacterial community to control hotspots of hydrolase activity in response to nitrogen supply. *Soil Biology and Biochemistry* 170:108717. doi: [10.1016/j.soilbio.2022.108717](https://doi.org/10.1016/j.soilbio.2022.108717).
- Henneron, L., P. Kardol, D. A. Wardle, C. Cros, and S. Fontaine. 2020. Rhizosphere control of soil nitrogen cycling: A key component of plant economic strategies. *New Phytologist* 228 (4):1269–82. doi: [10.1111/nph.16760](https://doi.org/10.1111/nph.16760).
- Herrmann, M. N., Y. Wang, J. Hartung, T. Hartmann, W. Zhang, P. M. Nkebiwe, X. Chen, T. Müller, and H. Yang. 2022. A global network meta-analysis of the promotion of crop growth, yield, and quality by bioeffectors. *Frontiers in Plant Science* 13:816438. doi: [10.3389/fpls.2022.816438](https://doi.org/10.3389/fpls.2022.816438).
- Khati, P., A. Sharma, S. Gangola, R. Kumar, P. Bhatt, and G. Kumar. 2017. Impact of agri-usable nanocompounds on soil microbial activity: An Indicator of soil health. *CLEAN – Soil, Air, Water* 45 (5):1600458. doi: [10.1002/clen.201600458](https://doi.org/10.1002/clen.201600458).
- Kubar, M. S., K. S. Alshallash, M. A. Asghar, M. Feng, A. Raza, C. Wang, K. Saleem, A. Ullah, W. Yang, K. A. Kubar, et al. 2022. Improving winter wheat photosynthesis, nitrogen use efficiency, and yield by optimizing nitrogen fertilization. *Life (Basel, Switzerland)* 12 (10):1478. doi: [10.3390/life12101478](https://doi.org/10.3390/life12101478).
- Li, B., T. Guo, and W. Zhou. 2022. Trehalose outperforms chitosan, humic acid and gamma-aminobutyric acid in promoting the growth of field maize. *Frontiers in Plant Science* 13:889615. doi: [10.3389/fpls.2022.889615](https://doi.org/10.3389/fpls.2022.889615).
- Li, B. Y., D. L. Song, T. F. Guo, X. P. Xu, C. Ai, and W. Zhou. 2022. Combined physiological and metabolomic analysis reveals the effects of different biostimulants on maize production and reproduction. *Frontiers in Plant Science* 13:1062603. doi: [10.3389/fpls.2022.1062603](https://doi.org/10.3389/fpls.2022.1062603).
- Li, J., T. van Gerrewey, and D. Geelen. 2022. A meta-analysis of biostimulants yield effectiveness in field trials. *Frontiers in Plant Science* 13:836702. doi: [10.3389/fpls.2022.836702](https://doi.org/10.3389/fpls.2022.836702).
- Liu, Z., K. Zhu, S. Dong, P. Liu, B. Zhao, and J. Zhang. 2017. Effects of integrated agronomic practices management on root growth and development of summer maize. *European Journal of Agronomy* 84:140–51. doi: [10.1016/j.eja.2016.12.006](https://doi.org/10.1016/j.eja.2016.12.006).
- Lynch, J., M. Cain, D. Frame, and R. Pierrehumbert. 2021. Agriculture's contribution to climate change and role in mitigation is distinct from predominantly fossil CO₂-emitting sectors. *Frontiers in Sustainable Food Systems* 4: 518093. doi: [10.3389/fsufs.2020.518039](https://doi.org/10.3389/fsufs.2020.518039).
- Marx, M. C., M. Wood, and S. C. Jarvis. 2001. A microplate fluorimetric assay for the study of enzyme diversity in soils. *Soil Biology and Biochemistry* 33 (12-13):1633–40. doi: [10.1016/S0038-0717\(01\)00079-7](https://doi.org/10.1016/S0038-0717(01)00079-7).
- Mattarozzi, M., J. Di Zinno, B. Montanini, M. Manfredi, E. Marengo, F. Fornasier, A. Ferrarini, M. Careri, and G. Visioli. 2020. Biostimulants applied to maize seeds modulate the enzymatic activity and metaproteome of the rhizosphere. *Applied Soil Ecology* 148:103480. doi: [10.1016/j.apsoil.2019.103480](https://doi.org/10.1016/j.apsoil.2019.103480).
- Medoro, V., G. Ferretti, G. Galamini, A. Rotondi, L. Morrone, B. Faccini, and M. Coltorti. 2022. Reducing nitrogen fertilization in olive growing by the use of natural chabazite-zeolite as soil improver. *Land* 11 (9):1471. doi: [10.3390/land11091471](https://doi.org/10.3390/land11091471).
- Mehrab, N., M. Chorom, and S. Hojati. 2016. Effect of raw and NH₄⁺-enriched zeolite on nitrogen uptake by wheat and nitrogen leaching in soils with different textures. *Communications in Soil Science and Plant Analysis* 47 (10):1306–16. doi: [10.1080/00103624.2016.1166377](https://doi.org/10.1080/00103624.2016.1166377).
- Miao, Y., D. J. Mulla, P. C. Robert, and J. A. Hernandez. 2006. Within-field variation in corn yield and grain quality responses to nitrogen fertilization and hybrid selection. *Agronomy Journal* 98 (1):129–40. doi: [10.2134/agronj2005.0120](https://doi.org/10.2134/agronj2005.0120).
- Moale, C., M. Ghiurea, C. G. Sirbu, R. Somoghi, T. M. Cioroianu, V. A. Faraon, C. Lupu, B. Trică, D. Constantinescu-Aruxandei, and F. Oancea. 2021. Effects of siliceous natural nanomaterials applied in combination with foliar fertilizers on physiology, yield and fruit quality of the apricot and peach trees. *Plants (Basel, Switzerland)* 10 (11):2395. doi: [10.3390/plants10112395](https://doi.org/10.3390/plants10112395).
- Mondal, M., B. Biswas, S. Garai, S. Sarkar, H. Banerjee, K. Brahmachari, P. K. Bandyopadhyay, S. Maitra, M. Brestic, M. Skalicky, et al. 2021. Zeolites enhance soil health, crop productivity and environmental safety. *Agronomy* 11 (3):448. doi: [10.3390/agronomy11030448](https://doi.org/10.3390/agronomy11030448).

- Morris, T. F., T. S. Murrell, D. B. Beegle, J. J. Camberato, R. B. Ferguson, J. Grove, Q. Ketterings, P. M. Kyveryga, C. A. M. Laboski, J. M. McGrath, et al. 2018. Strengths and limitations of nitrogen rate recommendations for corn and opportunities for improvement. *Agronomy Journal* 110 (1):1–37. doi: [10.2134/agronj2017.02.0112](https://doi.org/10.2134/agronj2017.02.0112).
- Mumpton, F. A. 1999. *La roca magica*: Uses of natural zeolites in agriculture and industry. *Proceedings of the National Academy of Sciences of the United States of America* 96 (7):3463–70. doi: [10.1073/pnas.96.7.3463](https://doi.org/10.1073/pnas.96.7.3463).
- Nephali, L., L. A. Piater, I. A. Dubery, V. Patterson, J. Huyser, K. Burgess, and F. Tugizimana. 2020. Biostimulants for plant growth and mitigation of abiotic stresses: A metabolomics perspective. *Metabolites* 10 (12):505. doi: [10.3390/metabo10120505](https://doi.org/10.3390/metabo10120505).
- Olander, L. P., and P. M. Vitousek. 2000. Regulation of soil phosphatase and chitinase activity by N and P availability. *Biogeochemistry* 49 (2):175–91. doi: [10.1023/A:1006316117817](https://doi.org/10.1023/A:1006316117817).
- Ordóñez, R. A., S. V. Archontoulis, R. Martínez-Feria, J. L. Hatfield, E. E. Wright, and M. J. Castellano. 2020. Root to shoot and carbon to nitrogen ratios of maize and soybean crops in the US Midwest. *European Journal of Agronomy* 120:126130. doi: [10.1016/j.eja.2020.126130](https://doi.org/10.1016/j.eja.2020.126130).
- Pantigoso, H. A., D. Newberger, and J. M. Vivanco. 2022. The rhizosphere microbiome: Plant-microbial interactions for resource acquisition. *Journal of Applied Microbiology* 133 (5):2864–76. doi: [10.1111/jam.15686](https://doi.org/10.1111/jam.15686).
- Petoumenou, D. G. 2023. Enhancing yield and physiological performance by foliar applications of chemically inert mineral particles in a rainfed vineyard under Mediterranean conditions. *Plants (Basel, Switzerland)* 12 (7):1444. doi: [10.3390/plants12071444](https://doi.org/10.3390/plants12071444).
- Roumani, A., and H. W. Olf. 2021. Nitrogen leaching and N use efficiency after application of zeolite and the nitrification inhibitor DMPP in a pot experiment with spring wheat. *Journal of Plant Nutrition* 44:1–10. doi: [10.1080/01904167.2021.1899217](https://doi.org/10.1080/01904167.2021.1899217).
- Rouphael, Y., and G. Colla. 2020. Toward a sustainable agriculture through plant biostimulants: From experimental data to practical applications. *Agronomy* 10 (10):1461. doi: [10.3390/agronomy10101461](https://doi.org/10.3390/agronomy10101461).
- Ruser, R., and R. Schulz. 2015. The effect of nitrification inhibitors on the nitrous oxide (N₂O) release from agricultural soils - a review. *Journal of Plant Nutrition and Soil Science* 178 (2):171–88. doi: [10.1002/jpln.201400251](https://doi.org/10.1002/jpln.201400251).
- Sanauallah, M., B. S. Razavi, E. Blagodatskaya, and Y. Kuzyakov. 2016. Spatial distribution and catalytic mechanisms of β -glucosidase activity at the root-soil interface. *Biology and Fertility of Soils* 52 (4):505–14. doi: [10.1007/s00374-016-1094-8](https://doi.org/10.1007/s00374-016-1094-8).
- Savarese, C., V. Cozzolino, M. Verrillo, G. Vinci, A. De Martino, A. Scopa, and A. Piccolo. 2022. Combination of humic biostimulants with a microbial inoculum improves lettuce productivity, nutrient uptake, and primary and secondary metabolism. *Plant and Soil* 481 (1-2):285–314. doi: [10.1007/s11104-022-05634-8](https://doi.org/10.1007/s11104-022-05634-8).
- Sedaghat, M., S. Hazrati, and M. Omrani. 2022. Use of zeolite and salicylic acid as an adaptation strategy against drought in wheat plants. *South African Journal of Botany* 146:111–7. doi: [10.1016/j.sajb.2021.10.001](https://doi.org/10.1016/j.sajb.2021.10.001).
- Sieradzki, E. T., E. E. Nuccio, J. Pett-Ridge, and M. K. Firestone. 2023. Expression of macromolecular organic nitrogen degrading enzymes identifies potential mediators of soil organic N availability to an annual grass. *The ISME Journal* 17 (7):967–75. doi: [10.1038/s41396-023-01402-3](https://doi.org/10.1038/s41396-023-01402-3).
- Sinaj, S., and W. Richner. 2017. Principes de fertilisation des cultures agricoles en Suisse (PRIF 2017). *Recherche Agronomique Suisse* 8:1–276.
- Siwik-Ziomek, A., and M. Szczepanek. 2019. Soil extracellular enzyme activities and uptake of N by oilseed rape depending on fertilization and seaweed biostimulant application. *Agronomy* 9 (9):480. doi: [10.3390/agronomy9090480](https://doi.org/10.3390/agronomy9090480).
- Struck, I. J., T. Reinsch, A. Herrmann, C. Klauß, R. Loges, and F. Taube. 2019. Yield potential and nitrogen dynamics of no-till silage maize (*Zea mays* L.) under maritime climate conditions. *European Journal of Agronomy* 107:30–42. doi: [10.1016/j.eja.2019.04.009](https://doi.org/10.1016/j.eja.2019.04.009).
- Tzanakakis, V. A., N. Monokrousos, and T. Chatzistathis. 2021. Effects of clinoptilolite zeolite and vermiculite on nitrification and nitrogen and phosphorus acquiring enzymes in a nitrogen applied agricultural soil. *Journal of Soil Science and Plant Nutrition* 21 (4):2791–802. doi: [10.1007/s42729-021-00566-1](https://doi.org/10.1007/s42729-021-00566-1).
- Trivedi, K., R. Kumar, K. G. Vijay Anand, G. Bhojani, D. Kubavat, and A. Ghosh. 2021. Structural and functional changes in soil bacterial communities by drifting spray application of a commercial red seaweed extract as revealed by metagenomics. *Archives of Microbiology* 204 (1):72. doi: [10.1007/s00203-021-02644-5](https://doi.org/10.1007/s00203-021-02644-5).
- Yakhin, O., A. A. Lubyantsev, I. Yakhin, and P. H. Brown. 2017. Biostimulants in plant science: A global perspective. *Frontiers in Plant Science* 7:2049. doi: [10.3389/fpls.2016.02049](https://doi.org/10.3389/fpls.2016.02049).
- Zadoks, J. C., T. T. Chang, and C. F. Konzak. 1974. A decimal code for growth stages of cereals. *Weed Research* 14 (6):415–21. doi: [10.1111/j.1365-3180.1974.tb01084.x](https://doi.org/10.1111/j.1365-3180.1974.tb01084.x).
- Zhang, L. L., G. Freschi, Y. Rouphael, S. De Pascale, and L. Lucini. 2023. The differential modulation of secondary metabolism induced by a protein hydrolysate and a seaweed extract in tomato plants under salinity. *Frontiers in Plant Science* 13:1072782. doi: [10.3389/fpls.2022.1072782](https://doi.org/10.3389/fpls.2022.1072782).
- Zhang, X., M. A. Dippold, Y. Kuzyakov, and B. S. Razavi. 2019. Spatial pattern of enzyme activities depends on root exudate composition. *Soil Biology and Biochemistry* 133:83–93. doi: [10.1016/j.soilbio.2019.02.010](https://doi.org/10.1016/j.soilbio.2019.02.010).