Effects of foliar application of a zeolite-based biostimulant on maize and winter wheat

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Maize leaves immediately after foliar treatment with the zeolite-based biostimulant. (Photo: Luca Bragazza, Agroscope)

Abstract

Among the challenges of agriculture in the coming decades is the necessity to increase crop yields and crop nutrient use efficiency while reducing the ecological footprint. Biostimulants can provide a solution to reach these goals. The aim of this study was to conduct a field test of the effects of foliar applications of a zeolite-based biostimulant on maize (three applications in 2022) and winter wheat (four applications in 2023) along a gradient of nitrogen fertilisation (50–155 kg N ha^{-1}). Maize silage biomass and wheat straw biomass, grain yields, thousand-kernel weights, and grain nutrient content were measured. Further, measurements of chlorophyll content and normalized difference vegetation index (NDVI) were taken during the growth of the two crops. We observed that the biostimulant increased wheat and maize yields at the lowest doses of nitrogen addition, particularly by increasing the number of grains per m^2. The increase in the harvest index seems to indicate an intensification of production. No effect on NDVI was observed, whereas chlorophyll content increased occasionally. The results also indicate an increase in the nitrogen use efficiency of wheat and maize, particularly at the lowest nitrogen fertilisation levels.

Keywords: yield, nitrogen, winter wheat, maize, zeolite

Introduction

The challenge of modern agriculture is to ensure food security for a growing world population while reducing the environmental footprints (Clark & Tilman, 2017; Lynch *et al.*, 2021). The use of plant biostimulants (PB) can represent a possible alternative for improving both nutrient use efficiency and abiotic stress tolerance in crops (Li *et al.*, 2022). Plant biostimulants are generally divided into two main categories. The first category includes BS of microbial origin, such as arbuscular mycorrhizal fungi[4] and plant growth-promoting rhizobacteria (Rouphael & Colla, 2020), while the second category includes PB of non-microbial origin. Different types of non-microbial PB can be distinguished, including chitosan, humic and fulvic acids, animal and plant protein hydrolysates, seaweed extracts, and silicon (Du Jardin, 2015; Sible *et al.*, 2021). Plant biostimulants can be coated on seeds, sprayed on plants (i.e. foliar application), or added to the soil.

The European Commission and the Swiss Federal Council have proposed a definition of biostimulants, which has since been included in the regulations on fertilisers (Regulation EU 2019; Le Conseil Fédéral Suisse, 2023). Here, regulations specify that plant biostimulants are products that stimulate plant growth and improve one or more additional functions, such as nutrient use efficiency, tolerance to abiotic stress, crop quality characteristics, and soil nutrient availability. In this case, two categories are distinguished depending on whether the PB is of microbial origin. Plant biostimulants have been used for decades, but in recent years, the offer has greatly increased and diversified. In fact, the biostimulant market is estimated to be worth USD 3.9 billion in 2023 and is expected to grow at an annual rate of around 12 % between 2023 and 2028 (Marketsandmarkets.com, 2024).

The category of inorganic biostimulants includes essential elements or inorganic salts, such as silicates and carbonates. Natural siliceous nanomaterials, such as zeolites, can be included in the inorganic PB category (Constantinescu-Aruxandei *et al.*, 2020). Zeolites (more than 200 known types) are aluminosilicates with a three-dimensional structure composed of interconnected $[SiO_4]^{4-}$ and $[AlO_4]^{5-}$ tetrahedra whose pores are generally occupied by exchangeable cations (Mumpton, 1999; Ramesh & Reddy, 2011). The internal surface area of zeolites can reach several hundred m^2 per gram, which explains their high cation exchange capacity. When applied as a soil amendment, natural zeolites can improve fertility (Akbari *et al.*, 2021; Faccini *et al.*, 2018; Ferretti *et al.*, 2024; Roumani & Olfs, 2021; Szatanik-Kloc *et al.*, 2021) and plant nutrition (Bernardi *et al.*, 2016; Medoro *et al.*, 2022; Mehrab *et al.*, 2016; Wu *et al.*, 2020). However, studies on the use of natural zeolite as PB by foliar application are sporadic (for example, Conversa *et al.*, 2024; El-Gabiery & Ata Allah, 2017; Moale *et al.*, 2021; Petoumenou, 2023) and, to our knowledge, no studies on field crops have been carried out.

A recent greenhouse experiment showed the positive effects of foliar application of a zeolite-based biostimulant for the plant and the soil in the case of winter wheat and maize (Quezada & Bragazza, 2024). Based on these results, the present study evaluates the effect of the foliar application of zeolite as a biostimulant on maize and winter wheat in the field along a gradient of nitrogen fertilisation during the years 2022 and 2023. The objectives are to test whether the application of a zeolite-based biostimulant can (1) increase grain yields and (2) improve nitrogen use efficiency, especially at lower levels of nitrogen fertilisation.

Materials and Methods

Study site and experimental design

The experiment was carried out at the experimental site of Agroscope in Nyon (46°24′05.28″N, 06°14′07.47″E, altitude 432 m) during the growing seasons 2022 and 2023. The climate at the study site is characterised by an average annual temperature of 11°C and annual precipitation of 1.004 mm. The soil is a Calcaric Cambisol with 200 g kg^{-1} of clay, 345 g kg^{-1} of sand, and a pH of 7.3 in the surface layer (0–20 cm). Maize (*Zea mays* L.) of the LG31226 variety was sown on April 28, 2022 and harvested on September 13, 2022 at a sowing density of 9.6 grains m^{-2} (row spacing = 75 cm). Thereafter, winter wheat (*Triticum aestivum* L.) of the Arina variety was sown on October 28, 2022 and harvested on July 13, 2023 at a sowing density of 450 grains m^{-2}.

The experimental design can be described as a split-plot design consisting of four blocks and 24 plots (5 m × 11 m). Two experimental factors were taken into account: the level of nitrogen fertilisation and foliar application of zeolite. Nitrogen was applied at four levels: 50 kg N ha^{-1}, 85 kg N ha^{-1}, 120 kg N ha^{-1} and 155 kg N ha^{-1}. Nitrogen was supplied in the form of ammonium nitrate (NH_4NO_3) for treatments at 50 kg N ha^{-1} and 120 kg N ha^{-1}, while it was supplied in the form of ammonium nitrate combined with cattle manure with a corresponding nitrogen input of 35 kg N ha^{-1} for treatments at 85 kg N ha^{-1} (50 + 35 kg N ha^{-1}) and 155 kg N ha^{-1} (120 + 35 kg N ha^{-1}).

For maize, four plots (replicates) were set up for each level of nitrogen fertilisation. For wheat, the N50 and N120 levels included eight plots, while the N85 and N155 levels included four plots. For each N level, zeolite (+Z) was applied by foliar application to half of the plot (5 m × 5.5 m, sub-plots), while the other half of the plot received the same amount of water without zeolite (Control or -Z). In total, the experimental design included eight treatments (50-Z, 50+Z, 85-Z, 85+Z, 120-Z, 120+Z, 155-Z, 155+Z) with a total of 32 and 48 sub-plots for maize and winter wheat, respectively. For winter wheat, three applications of N and four applications of zeolite were provided (Fig. 1), whereas two applications of N and three applications of zeolite were provided for maize. For potassium and phosphorus fertilisation, triple superphosphate 46 % P_2O_5 [$Ca(H_2PO_4)$] and granular potassium 60 % K_2O [KCl] were used as fertilisers in accordance with fertilisation standards for winter wheat and maize (Sinaj *et al.*, 2017).

Foliar application of the zeolite-based biostimulant

The biostimulant used in this study was the FertiRoc® product, a biostimulant based on a natural zeolite (chabazite) mixed with a proportion of natural soft calcium carbonate. The composition is micronised and extremely finely processed using the know-how of the manufacturer, Power the Nature SA (Lausanne-Switzerland and Paris-France). The FertiRoc© product has been validated by a certification agency and accepted as a plant biostimulant according to the EU regulation 2019/1009, implying that the biostimulant characteristics of the product are not linked to its nutrient content. The zeolite powder was mixed with water and applied by hand using a motor sprayer. The biostimulant was applied four times for winter wheat and three times for maize. The corresponding doses for the winter wheat were: 1.5, 1.5, 2.0, and 2.0 kg FertiRoc©/ha/300L of water. The first three applications followed nitrogen ap-

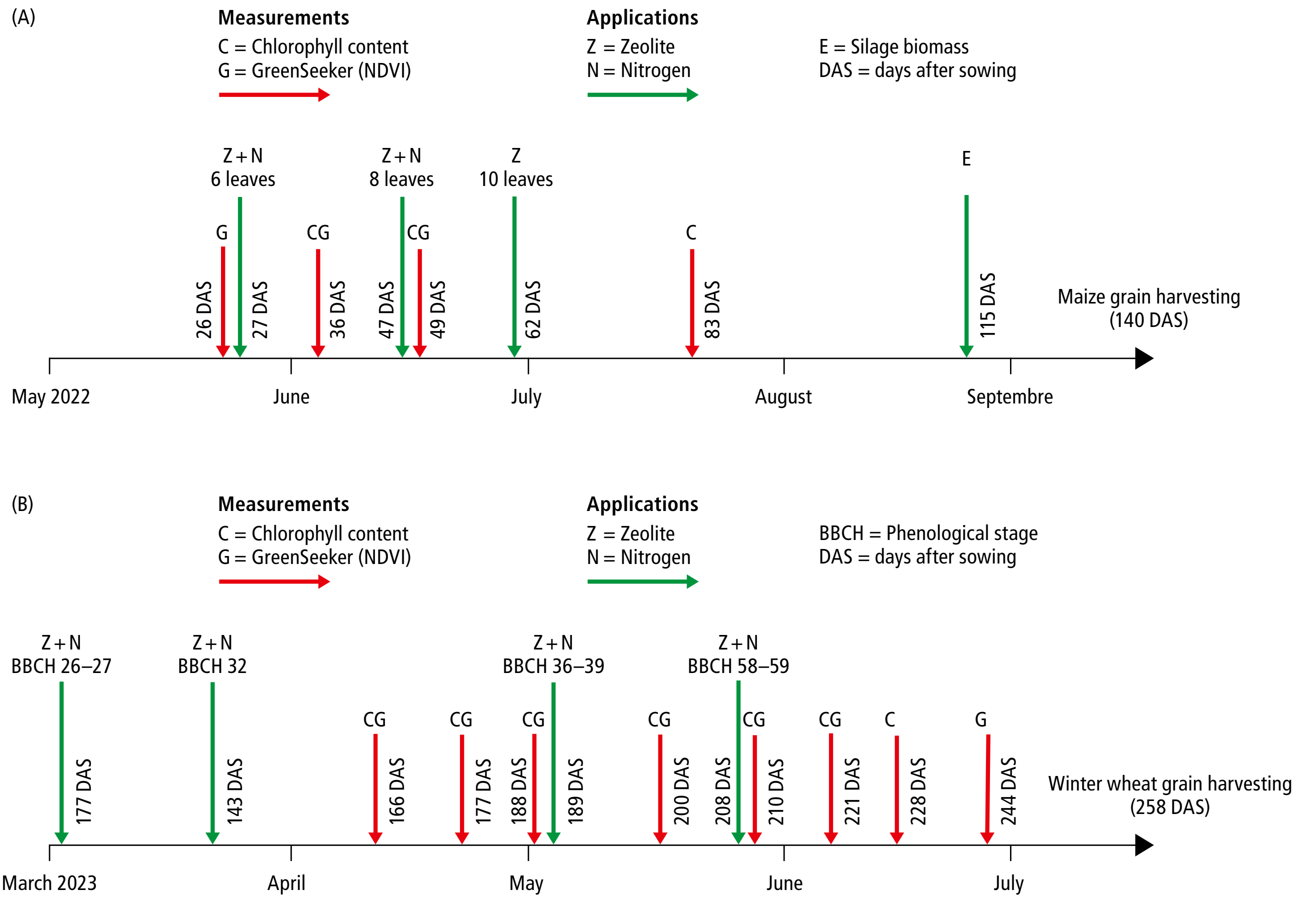

Figure 1 | Indication (green arrows) of the date of nitrogen (N) fertilisation and foliar applications of the zeolite-based biostimulant (Z) during the 2022 growing season for maize and the 2023 season for winter wheat. The red arrows indicate the dates of measurements of leaf chlorophyll content (C) and NDVI (G). The number of days after the sowing date (April 28, 2022 for maize and October 28, 2022 for winter wheat) is indicated (DAS) for each agronomic intervention and each measurement, while the phenological stages (BBCH scale) refer to the application dates of the biostimulant.

plications at the following phenological stages (BBCH): 26–27, 32, 36–39. A fourth application was made at BBCH 58–59. For the maize, the three foliar applications were made at the six-leaf stage (BBCH 16), eight-leaf stage (BBCH 18), and ten-leaf stage (BBCH 19), and the corresponding biostimulant doses were 3.0, 3.0, and 2.0 kg Fertiroc®/ha/300L water. Foliar applications were systematically followed by at least two rain-free days.

Periodical non-destructive field measurements

The leaf chlorophyll content (Fig. 1) was periodically measured using a CL-01 chlorophyll meter (Hansatech Instruments) on the last fully developed leaf of four wheat plants and two maize plants representative of each plot. The GreenSeeker instrument (Trimble) was used for periodical normalised difference vegetation index (NDVI) measurements by 'scanning' the surface of each plot using four measurements and keeping the instrument at a distance of around 60 to 80 cm from the crop (Fig. 1). The average NDVI was then calculated for each plot.

Grain harvesting, plant biomass, and nutrient analysis

For maize, the harvesting of silage biomass took place on August 17, 2022 over a surface of 1 m^2, whereas the grain harvesting was carried out on September 13, 2022 using an experimental harvesting machine on a central strip 2.25 m wide in each plot (5.5 m long). For winter wheat, the grain and straw biomass were harvested on July 13, 2023 using an experimental harvesting machine on a central strip 2.2 m wide in each plot (5.5 m long). The yield was expressed at a standard moisture content of 14.0 % for maize and 14.5 % for winter wheat. The grain N concentration was determined using the Dumas method (NF ISO 13 878). The other macronutrients, that is, calcium, magnesium, potassium, and phosphorus, were determined after mineralisation with aqua regia, followed by inductively coupled plasma-mass-spectrometry (ICP-MS) analysis.

Statistical analysis

Statistical analyses were carried out using the R 3.01 program (Team, 2013). For each fertilisation level, data from treatments with and without the biostimulant were compared using paired t-tests for each plot (to take into account field heterogeneity) using the t-test function.

Results and Discussion

For the lowest nitrogen fertilisation rate (50 kg N ha^{-1}), foliar application of the biostimulant increased grain yields by 7 % for winter wheat and 5 % for maize (Fig. 2). For the N fertilisation rate of 85 kg N ha^{-1}, we observed an increase in grain yield of 6 % for winter wheat and 9 % for maize, with significant responses for both crops. For N fertilisation rates of 120 kg N ha^{-1} and 155 kg N ha^{-1}, wheat yield increased by 7 % and 6 %, respectively, while no effect on grain yield was observed for maize. Our results suggest that (1) the grain yield response to the biostimulant is crop-specific, with winter wheat appearing to be more responsive than maize, and (2) the effect of the biostimulant is greater at lower N application rates. Although several studies have highlighted an increase in productivity in response to the application

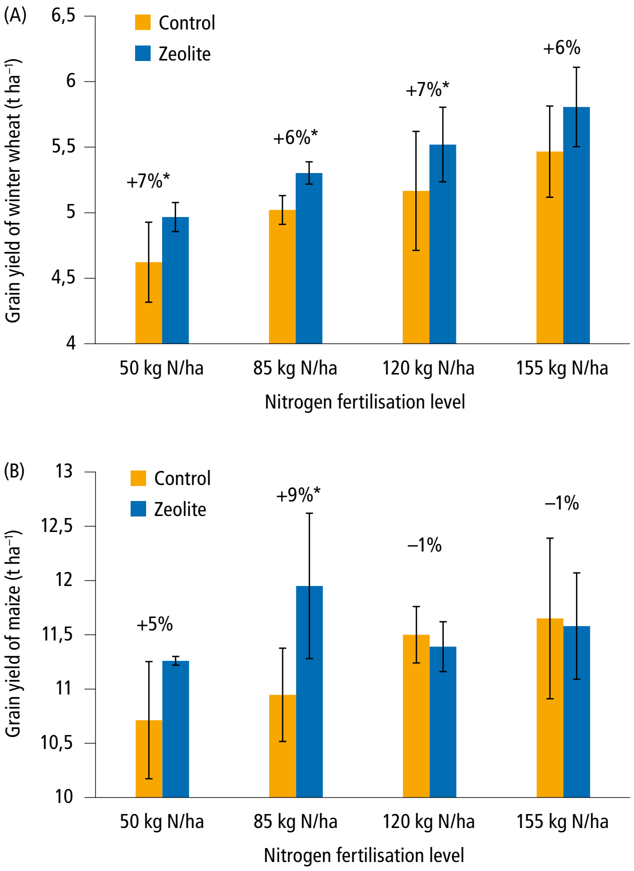

Figure 2 | **Mean (± standard deviation) of grain yield of winter wheat (A) and maize (B) for the four levels of N fertilisation in response to the foliar application of the zeolite-based biostimulant. The mean percentage difference between the control and treatment groups is also indicated. The asterisk indicates a significant difference ($p < 0.05$) between the control and the treatment for each level of N fertilisation.**

of biostimulants (Bulgari *et al.*, 2015; Del Buono, 2021; Drobek *et al.*, 2019; Li *et al.*, 2022), to our knowledge, there are still no data on the response of field crops to foliar application of a zeolite-based biostimulant.

The foliar application of the biostimulant also increased the number of winter wheat grains per m^2 by 6 %, on average, across the entire N fertilisation gradient (Fig. 3). The fact that the applications were made after tillering (Fig. 1) suggests that this outcome was due to the increase in the number of grains per spike, not the number of spikes per m^2, in accordance with the reported effect of other foliar biostimulants that can increase the number of grains per spike in winter wheat (Szczepanek & Grzybowski, 2016). Similarly, the number of grains per m^2 in the maize trial increased by 7.9 % and 9.3 % for N fertilisation rates of 50 kg N ha^{-1} and 85 kg N ha^{-1} but, unlike wheat, not significantly (Fig. 3).

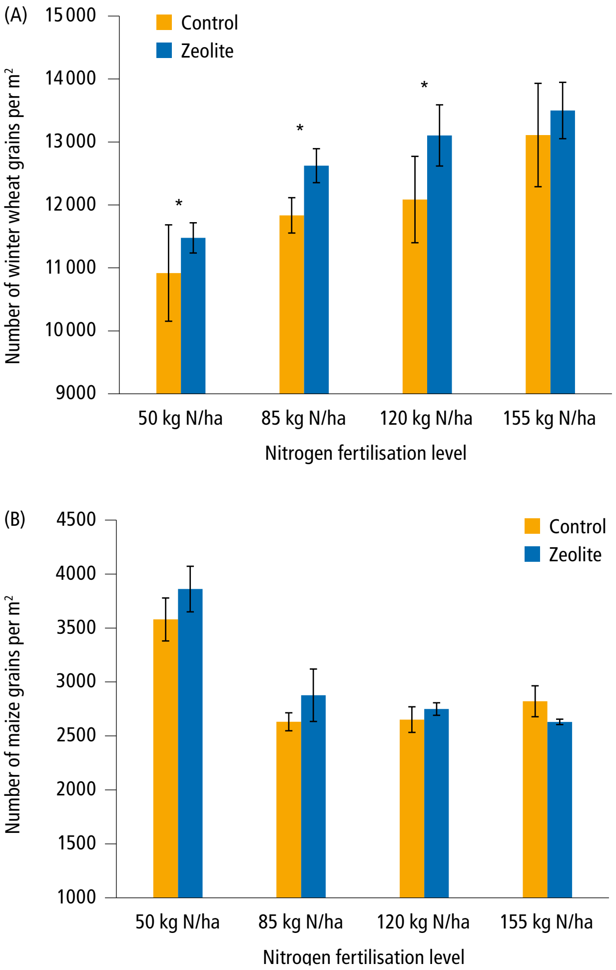

Figure 3 | Mean (± standard deviation) number of grains per m^2 of winter wheat (A) and maize (B) for the four different levels of N fertilisation in response to the foliar application of the zeolite-based biostimulant. The asterisk indicates a significant difference ($p < 0.05$) between control and treatment for each level of N fertilisation.

The increase in grain yield following the application of the biostimulant did not lead to a decrease in thousand-kernel weight for either winter wheat or maize (Table 1), as generally observed in other studies following an increase in the number of kernels per m^2 (Acreche & Slafer, 2006; Lenoir *et al.*, 2023). By contrast, for the fertilisation rate of 50 kg N ha^{-1}, the increase in wheat yield due to the biostimulant was also favoured by a significantly higher thousand-kernel weight (Table 1), in addition to the increase in the number of grains per m^2 (Fig. 3). The lower number of wheat kernels per m^2 with 50 kg N ha^{-1} compared to higher doses of N fertilisation may have reduced the competition between kernels and thus facilitated an increase in the thousand-kernel weight following the application of the biostimulant.

The application of the zeolite-based biostimulant reduced winter wheat straw biomass production by 4 % on average across the N fertilisation gradient, and this reduction was significant at 85 kg N ha^{-1} and 155 kg N ha^{-1} (Table 1). However, the application of the biostimulant had no effect on silage biomass production for maize (Table 1).

Over the entire fertilisation gradient, the winter wheat harvest index increased from an average value of 0.45 in the controls to 0.48 after the application of the FertiRoc© biostimulant, corresponding to an increase of around 6.3 % (Fig. 4). A more substantial increase was observed for the lower levels of N fertilisation (50 kg N ha^{-1} and 85 kg N ha^{-1}), resulting in a higher relative increase in grain yield and a more distinct reduction in straw production than for the higher levels of fertilisation (Fig. 2, Table 1). For maize, the harvest index increased (non-significantly) by a smaller amount, from 0.47 in the controls to 0.48 with the addition of the biostimulant (Fig. 4). The increase in the harvest index is often linked to an increase in distal florets (Evans & Fischer, 1999). By comparison, the foliar application of zeolite to the coriander increased the number of umbels per plant as well as their height (Mahmoud *et al.*, 2023). A more in-depth study of the influence of zeolite on the number of flowers per spike, spike growth, and grain distribution per spike would be necessary to show how the foliar application of the biostimulant modifies the yield components.

No change in N concentration in the grains was observed following the application of the zeolite-based biostimulant either for winter wheat or maize (Table 2). Thus, even when grain yield increased (Fig. 2), there was no dilution of the N concentration, an important result with regard to the qualitative value (i.e. protein content) of the winter wheat and maize grains. For maize, dilutions of P, K, Ca, and Mg concentrations were observed only at the lowest fertilisation dose (50 kg N ha^{-1}). However, no dilution of macronutrients in the grains was observed in winter wheat. Surprisingly, the 120 kg N ha^{-1} fertilisation level combined with zeolite was characterised by a significant increase in K, Ca, and Mg content ($p < 0.05$) in winter wheat grains (Table 2).

The amount of N exported by winter wheat grains increased by 6.5 % across the N fertilisation gradient (Fig. 5), while an increase of 3 % and 10 % was observed for maize grains at fertilisation levels of 50 kg N ha^{-1} and 85 kg N ha^{-1}, respectively. Similarly, an increase in export has already been shown in a greenhouse experiment with winter wheat and maize (Quezada & Bragazza, 2024). Increased N export may have important environmental implications if it reduces the loss of N from chemical fertilisers. Furthermore, for the winter wheat fertilised at 120 kg N ha^{-1} and for the maize fertilised at 85 kg N ha^{-1}, significant increases ($p < 0.05$) in the export of P (+9.1 % and +13.9 %), K (+10.8 % and +13.7 %), and Mg (+10.2 % and +16.2 %) were observed. These results suggest that nutrient uptake as a whole can be enhanced by the zeolite-based biostimulant. For example, for the entire N fertilisation gradient, the biostimulant enabled wheat grains to export 7.59 kg N ha^{-1}, 1.58 kg P ha^{-1}, and 1.67 kg K ha^{-1} more than the control, whereas only 5 g N ha^{-1}, 7.7 g P ha^{-1}, and 83 g K ha^{-1} were supplied directly by the biostimulant. These results suggest that, potentially, a reduction in fertiliser amount could be achieved by improving the uptake efficiency through the use of a biostimulant. To our knowledge, the effect of zeolite on the uptake of nutrients other than N has never been studied. Other studies have shown that foliar application of amino acid-based biostimulants in association with microelements can increase the concentration of Cu, Na, Ca, and Mo in winter wheat grains, in addition to increasing grain yield by an order of magnitude similar to our observations (Popko *et al.*, 2018).

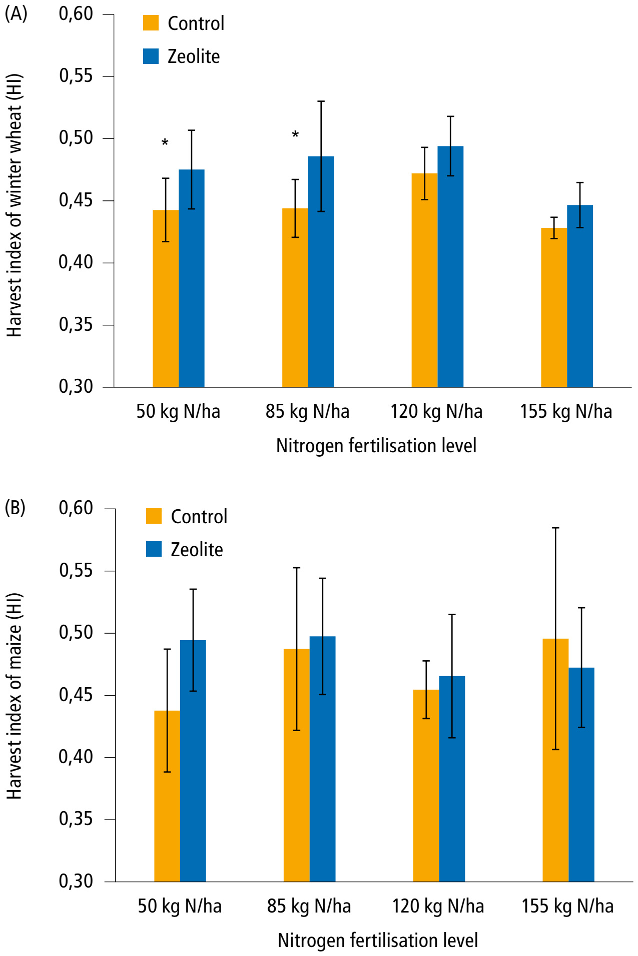

Figure 4 | Mean value (± standard deviation) of the harvest index of winter wheat (A) and maize (B) for the four different levels of N fertilisation in response to the foliar application of the biostimulant. The asterisk indicates a significant difference ($p < 0.05$) between the control and the zeolite treatment for each level of N fertilisation.

To elucidate the mechanisms of action of the biostimulant, numerous non-destructive measurements related to crop photosynthesis were carried out during the growing season (Fig. 1). Periodic NDVI measurements showed no significant differences between treatments

Table 1 | Thousand-kernel weight, straw biomass of winter wheat, and silage biomass of maize for the four levels of nitrogen (N) fertilisation in the controls and in the treatments with the zeolite-based biostimulant. Letters indicate a significant difference ($p < 0.05$) after comparison by t-test.

N fertilisation level	Winter wheat (year 2023)				Maize (year 2022)			
	Thousand-kernel weight (g)		Straw (t ha^{-1})		Thousand-kernel weight (g)		Silage biomass (t ha^{-1})	
	Control	Zeolite	Control	Zeolite	Control	Zeolite	Control	Zeolite
N = 50 kg ha^{-1}	42.4 B	43.3 A	5.9 A	5.6 A	299 A	293 A	24.8 A	23.0 A
N = 85 kg ha^{-1}	42.4 A	42.0 A	6.4 A	5.8 B	416 A	417 A	22.8 A	24.2 A
N = 120 kg ha^{-1}	42.7 A	42.1 A	5.8 A	5.7 A	435 A	414 B	25.5 A	24.8 A
N = 155 kg ha^{-1}	41.7 A	43.0 A	7.3 A	7.2 B	413 B	440 A	24.3 A	25.2 A

Table 2 | Effect of the foliar application of the zeolite-based biostimulant on the mean concentration of macronutrients (i.e. nitrogen, phosphorus, potassium, calcium, and magnesium) in winter wheat and maize grains for the four different N fertilisation levels. Letters indicate a significant difference ($p < 0.05$).

Crop	N fertilisation level	N (%)		P (%)		K (%)		Ca (‰)		Mg (%)	
		Control	Zeolite	Control	Zeolite	Control	Zeolite	Control	Zeolite	Control	Zeolite
Winter wheat	N = 50 kg ha^{-1}	2.04 A	2.01 A	0.420 A	0.425 A	0.495 A	0.456 A	0.534 A	0.540 A	0.124 A	0.126 A
	N = 85 kg ha^{-1}	2.06 A	2.04 A	0.432 A	0.424 A	0.454 A	0.441 A	0.528 A	0.530 A	0.129 A	0.127 A
	N = 120 kg ha^{-1}	2.29 A	2.34 A	0.424 B	0.436 A	0.427 B	0.447 A	0.561 B	0.589 A	0.125 B	0.131 A
	N = 155 kg ha^{-1}	2.27 A	2.27 A	0.421 A	0.412 A	0.433 A	0.415 A	0.565 A	0.560 A	0.124 A	0.123 A
Maize	N = 50 kg ha^{-1}	1.25 A	1.23 A	0.243 A	0.226 B	0.394 A	0.379 B	0.105 A	0.080 B	0.968 A	0.900 B
	N = 85 kg ha^{-1}	1.30 A	1.32 A	0.212 A	0.221 A	0.350 A	0.363 A	0.073 A	0.068 A	0.843 A	0.090 B
	N = 120 kg ha^{-1}	1.33 A	1.32 A	0.240 A	0.221 A	0.379 A	0.364 A	0.080 A	0.073 A	0.970 A	0.885 A
	N = 155 kg ha^{-1}	1.36 A	1.37 A	0.218 A	0.221 A	0.365 A	0.365 A	0.070 A	0.070 A	0.900 A	0.925 A

with and without the biostimulant for either winter wheat or maize (data not shown). The foliar chlorophyll content increased significantly ($p < 0.05$) only occasionally during the study period for the two crops in response to the biostimulant, that is, only for one date and for one treatment. By contrast, the application of zeolite to coriander was shown to increase its chlorophyll content (Mahmoud *et al.*, 2023). Overall, the effect of zeolite application on photosynthesis is very poorly understood and remains to be elucidated. It is possible that the biostimulant can influence crops through a combination of "direct" effects linked to the deposition of zeolite on the leaves and "indirect" effects linked to a change in the crop's metabolism (Quezada & Bragazza, 2024).

Conclusion

Overall, the results of this first field trial of foliar applications of FertiRoc© product as a zeolite-based biostimulant in combination with different doses of N fertilisation seem promising. Data collected for the years 2022 and 2023 at the Agroscope-Nyon research station showed an average increase in winter wheat grain yield (Arina variety) of 6.7 % along the entire N fertilisation gradient (from 50 to 155 kg N ha^{-1}) and an increase in maize yield (LG31226 variety) of 7.2 % for the lowest nitrogen fertilisation doses (50 and 85 kg N ha^{-1}). For these same N fertilisation rates, the harvest index also increased by 6.3 % for winter wheat and by 7.2 % for maize. We also recorded an increase in N export in the grains because no dilution of nutrient concentration was observed following the application of the biostimulant. These results indicate an increase in N use efficiency for

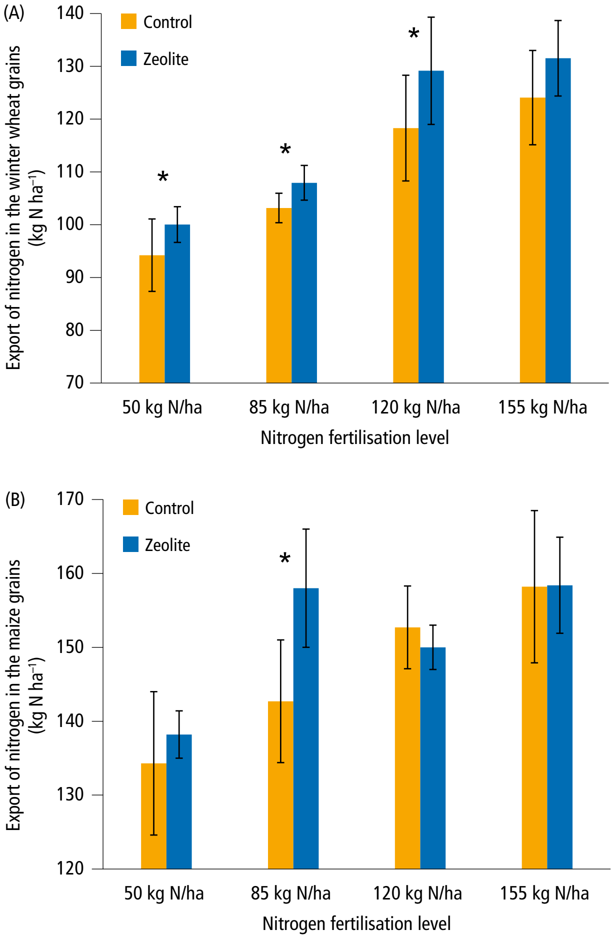

Figure 5 | Mean value (± standard deviation) of nitrogen export in winter wheat grains (A) and maize grains (B) for the four different levels of nitrogen fertilisation in response to foliar application of the zeolite-based biostimulant. The asterisk indicates a significant difference ($p < 0.05$) between the control and the zeolite treatment for each level of nitrogen fertilisation.

winter wheat and maize, especially at the lowest N fertilisation levels. Higher N use efficiency may suggest the possibility of reducing the amount of N fertiliser applied to a crop after the foliar application of the biostimulant while maximising N export, with the additional advantage of reducing the environmental losses of N. However, future studies are needed to confirm these results under other soil and climatic conditions and for other crops, as well as to gain a better understanding of the metabolic and ecophysiological responses to biostimulant applications. Finally, a technical and economic assessment of the use of the biostimulant would make it possible to compare, depending on the crop and the site, the economic gain from a reduction in N fertilisation and/or an increase in productivity with the cost of applying the biostimulant. ■

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