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The Integrated Effects of Biostimulant Application, Mechanical Weed Control, and Herbicide Application on Weed Growth and Maize (*Zea mays* L.) Yield

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Abstract: A field trial was conducted (2020–2021) in a randomized complete block design arranged according to the split-plot design to evaluate the integrated effects of an alternative fertilization practice based on the application of a microbial biostimulant in combination with different weed control methods on weed growth and maize productivity. Two fertilization practices, conventional (CF) and alternative (AF), formed the main plots. The CF supplied maize with 160 kg N ha^{-1}. The AF included a foliar application of the biostimulant NitroStim®, which contains N_2-fixing bacteria (1×10^{12} colony forming units; CFU L^{-1}) along with a 50% lower fertilizer incorporation rate (80 kg N ha^{-1}). Four weed control treatments formed the subplots: one inter-row mechanical cultivation (M1), two inter-row mechanical cultivations (M2), tembotrione application (99 g a.i. ha^{-1}; H), and an untreated control (CON). Combined over the years ($p \geq 0.05$), fertilization, weed control, and their interactions affected ($p \leq 0.05$) weed density and biomass, maize grain yield, and nitrogen partial factor productivity (PFP_N). The AF reduced weed biomass by 28% compared to the CF. M1 resulted in a high value (389 g m^{-2}). M2 and H reduced weed biomass compared to (M1 $\geq$ 70%). Weed biomass dropped below 35 g m^{-2} in the AF × H and AF × M2 subplots. Observations on weed density were similar. The AF resulted in 12 and 56% higher maize grain yield and PFP_N than the CF, respectively. M2 increased grain yield by 18 and 25% compared to M1 and CON, respectively, and was not different from H. Moreover, AF × H and AF × M2 were the highest-yielding interactions ($\geq$12,000 kg grain ha^{-1}). AF × M2 increased PFP_N by 56, 58, 64, and 67% compared to CF × H, CF × M2, CF × M1, and CF × CON, respectively, while AF × H resulted in similar PFP_N.

Keywords: NitroStim®; nitrogen partial factor productivity (PFP_N); nitrogen fertilization; inter-row cultivation; tembotrione

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1. Introduction

Weed management is an essential agronomic practice in the cultivation of maize (*Zea mays* L.; 2*n* = 2*x* = 20; *Poaceae*), the world's third most important cereal crop after wheat and rice [1]. Weed competition is the most important barrier to achieving higher yields in maize cropping systems, resulting in severe yield losses of up to 60% worldwide [2].

However, nowadays, in the era of climate change, developing effective weed management systems is more challenging than ever [3]. This is because current and projected increases in atmospheric concentrations of CO_2 and other radiatively active gases are affecting important climate variables such as temperature, precipitation, relative humidity, radiation, etc., and subsequently weed and crop growth and weed-crop interactions [4].

Weeds respond directly to climate change and exhibit better survival mechanisms than domesticated crops due to their interspecific genetic variation, leading to physiological and phenotypic plasticity, which increases their adaptive capacity [5]. For example, higher concentrations of atmospheric CO_2 stimulate photosynthesis and the growth of C_3 weeds and reduce stomatal aperture and increase water use efficiency in both C_3 and C_4 weeds [6]. In addition, the effects of environmental factors on the performance of herbicides, whose use remains the most common method of weed control in modern agriculture, are expected to become uncertain and unpredictable [5]. Therefore, to meet the challenges of this new era, multiple non-chemical and chemical weed control methods need to be integrated into more diverse weed management systems [7].

For the development of effective and sustainable integrated weed management systems for maize and other economically important arable crops, fertilization is a critical factor that must be considered. In many cases, fertilizer use can favor weed growth over crop growth and give weeds a competitive advantage [8]. This is especially true for base fertilization with high nitrogen inputs to the soil during the critical early growth stages of the crop, when weeds can grow aggressively and take up nutrients faster than the crop, displacing young crop plants and hindering canopy establishment [9]. Conventional broadcast nitrogen fertilization generally increases weed pressure compared to more innovative alternative fertilization practices that aim to reduce the amount of nitrogen available to weeds in the soil [10]. Consequently, alternative fertilization practices should be considered important for weed management. Although band fertilization, i.e., the precise placement of fertilizers in bands under crop rows, is the most studied method for such purposes [11–14], the role of non-chemical plant biostimulants in developing sustainable fertilization and weed management strategies should also be investigated.

In general, there is growing interest in the role of non-chemical plant biostimulants in the development of alternative fertilization practices that could facilitate the shift of agriculture to more environmentally friendly approaches while reducing weed pressure on crops [15]. Plant biostimulants are formulated products of a biological origin such as natural compounds, enzymes, plant growth regulators, and microorganisms (fungi, bacteria, etc.) that improve plant productivity due to the novel or emerging properties of their complex of constituents [16]. Bacterial biostimulants are a very important category of plant biostimulants, and their most prominent group includes plant growth-promoting bacteria that colonize the rhizosphere of plants [17]. A major category of beneficial bacteria consists of N_2-fixing bacterial species belonging to *Rhizobium* spp., *Azotobacter* spp., *Azospirillum* spp., *Pseudomonas* spp., and *Bacillus* spp. [18]. These bacteria convert atmospheric nitrogen (N_2) into ammonia (NH_3) through the process of biological nitrogen fixation, a source of nitrogen that becomes available to crops [19].

Therefore, the application of biostimulants containing N_2-fixing bacteria is one of the most attractive alternatives to the excessive use of inorganic nitrogen fertilizers. In addition, the use of biostimulants is expected to have a positive effect on weed control, as shown by recent studies. For example, Soltani et al. [20] reported an excellent efficacy of the combined applications of herbicides and biostimulants on *Abutilon theophrasti* Medik., *Amaranthus retroflexus* L., *Ambrosia artemisiifolia* L., *Chenopodium album* L., and *Setaria viridis* and other annual grasses in field trials with maize. Similar results were reported in the study by Matysiak et al. [21] in spring wheat. In potato, herbicide application with biostimulants had a positive effect on weed control and increased marketable yields by about 25% [22].

However, the above research results are from studies that did not test biostimulant products containing N_2-fixing bacteria and instead examined other categories of biostimulants. In the absence of relevant studies with this particular category of biostimulants, the objective of the current study was to evaluate the integrated effects of alternative fertilization practices based on biostimulants containing N_2-fixing bacteria in combination with mechanical weed control and herbicide application on weed growth and the productivity of maize under real field conditions.

2. Materials and Methods

2.1. Site Description

A field trial was conducted during the spring and summer months of 2020 and 2021 to evaluate different fertilization practices and weed control treatments in a maize field in western Greece, about 90 km northwest of Agrinio (latitude 20°53′54″ east (E), longitude 38°53′38″ north (N)). The soil type was clay loam(CL), with the following texture and physicochemical properties (0–15 cm): clay 296 g kg^{-1}, silt 337 g kg^{-1}, sand 367 g kg^{-1}, organic matter 14.4 g kg^{-1}, pH (1:2 H_2O) 7.6, and $CaCO_3$ 12 g kg^{-1}. The monthly average temperature increased in all months of 2021 compared to those of 2020. In addition, lower precipitation heights fell in the second growing season compared to those in 2020, and this was observed for all months except August (Table 1).

Table 1. Monthly average temperature (°C) and precipitation height (mm) in the experimental area for both growing seasons (2020 and 2021).

Month	Average Temperature (°C)		Total Precipitation (mm)	
	2020	2021	2020	2021
April	14.8	15.1	39.8	10.2
May	21.0	21.2	48.8	2.8
June	23.2	25.0	25.6	20.6
July	28.1	29.4	24.2	0.0

Datura stramonium L., *Amaranthus retroflexus* L., *Chenopodium album* L., *Solanum nigrum* L., *Xanthium strumarium* L., *Polygonum aviculare* L., and *Portulaca oleracea* L. were the most common broadleaf weeds while *Setaria* spp., especially *Setaria viridis* (L.) P. Beauv. and *Setaria faberi* Herrm., were the dominant grass weed species. Alfalfa (*Medicago sativa* L.) was grown at this site during the past four years [19].

2.2. Experimental Setup and Design

The experiment was laid out in a randomized complete block design (RCBD) with four replicates arranged according to the split−plot design with two experimental factors. Two fertilization practices, conventional and alternative, were assigned to the main plots. Four weed control treatments were assigned to subplots. There was a total of two main plots and 32 subplots. The subplots were 6 m^2 (2 m long × 3 m wide), and the main plot size was 96 m^2 (2 m long × 3 m wide), giving a total experimental area of 192 m^2. Weed-free boundaries of 0.75 m were maintained between adjacent subplots and 1.5 m between the two main plots.

The soil was ploughed in the fall (30 cm deep) and disked (20 cm deep) before sowing to break up the clods of soil and prepare the seedbed. In the second year, different plots were established to actually repeat the experiment in time. The sown plant material was the medium-early maize hybrid (FAO 500) '*P0937*' (Pioneer Hi-Bred Hellas S.A., Athens, Greece) with a maturity time of 120–125 days. Sowing was performed on 14 April 2020 and 10 April 2021 with a sowing rate of 91,000 seeds ha^{-1}, resulting in a density of 85,000 plants ha^{-1}. The row spacing was 75 cm, and the sowing depth was 5 cm. A sprinkler (Grouner 1938, Demiroglou, Sot., & Sons O.E., Grouner—Dischargers of Artificial Rain, Thessaloniki, Greece) was placed at the boundary between the two main plots to irrigate the field and maintain soil moisture during the crucial growth stages of the crop, depending on the rainfall that fell during the two growing seasons, to provide the maize plants with a total of 600 mm of water in both 2020 and 2021.

The conventional fertilization practice (CF) involved the incorporation of a granular nitrogen fertilizer applied at a rate of 765 kg ha^{-1} with a nitrification inhibitor (Slowtec Plus® 21-0-0, Phytothreptiki S.A., Aspropirgos, Greece) to provide 160 kg N ha^{-1} to the crop. The alternative fertilization practice (AF) involved foliar application of the biostimulant NitroStim® (Humofert S.A., Athens, Greece) along with a 50% lower fertilizer rate (381 kg of product ha^{-1}) to supply the maize plants with 80 kg N ha^{-1}. NitroStim® is a prepackaged microbial solution containing N_2-fixing bacteria at a concentration of 1 × 10^{12} colony forming units (CFU) L^{-1}. The biostimulant was applied at the 3-leaf growth stage of maize (BBCH: 13) using an Elettra Venus™ 5 pressure sprayer (Viopsec Kalimeris SMPC, Athens, Greece) calibrated to deliver 5 L ha^{-1} of spray solution at a constant pressure of 200 kPa through a brass conical nozzle (Viopsec Kalimeris SMPC, Athens, Greece). The dates for biostimulant application were 4 May 2020 and 2 May 2021.

Weed control treatments included: one inter-row mechanical cultivation (M1) at the 4-leaf growth stage of maize (BBCH: 14), two inter-row mechanical cultivations (M2) at the 4- and 6-leaf growth stages of maize (BBCH: 14 and 16), and the application of tembotrione at the 6-leaf growth stage of maize (BBCH: 16). For the M1 treatment, the mechanical passes were conducted on 11 May 2020 and 8 May 2021. For the M2 treatment, the first mechanical passes were conducted on 11 May 2020 and 8 May 2021, and the second passes were conducted on 25 May 2020 and 22 May 2021. Tembotrione (Laudis® WG, Bayer Hellas S.A., Athens, Greece) was applied at a rate of 99 g a.i. ha^{-1} using a Gloria® 405 T (Gloria Haus & Gartengeraete GMBH, Witten, Germany) pressurized sprayer equipped with a 2.4 m wide boom and six TeeJet® 8002flat fan nozzles (TeeJet Technologies Northwest Europe, Schorndorf, Germany) calibrated to deliver 300 L ha^{-1} of spray solution at a constant pressure of 250 kPa (H). The dates of herbicide application were 25 May 2020 and 22 May 2021. An untreated control was also included (CON).

Pyraclostrobin, a strobilurin fungicide (Comet® 20 EC, Basf Hellas S.A., Athens, Greece), was also applied in both growing seasons at the beginning of stem elongation (BBCH: 30). Fungicide applications were performed with the same equipment as described above at an application rate of 200 g a.i. ha^{-1} against the fungal species causing maize leaf blight, namely *Helminthosporium turcicum* Pass. (1876). The sprayer was calibrated to deliver 400 L ha^{-1} of spray solution at a constant pressure of 280 kPa. No infestation of insect pests was observed in either growing season.

2.3. Data Collection

In both years, weed density and biomass were assessed on 30 May and 15 July, respectively. To measure weed density, weeds were counted in two 1 m^2 wooden quadrats randomly placed near the center of each subplot away from the edges in areas of uniform weed flora composition. These areas were marked with 1 m high wooden stakes to measure weed biomass 45 days later. For these measurements, weeds were cut with scissors at a height of 2–3 cm, placed in numbered plastic bags, and brought to the laboratory. Samples were oven-dried at 65 °C for 48 h (DHG-9025, Knowledge Research S.A., Athens, Greece) and measured using an electronic balance with three decimal places (KF-H2, Zenith S.A., Athens, Greece). On 28 August 2020 and 22 August 2021, at the stage of maize grain maturity (BBCH: 89), ears from 12 plants in the middle rows of each subplot were harvested by hand in two areas of 1 m^2 in each subplot and dried in the oven at 70 °C until a constant weight was reached. Grain yield per unit area was then determined by multiplying the number of ears per unit area, the number of rows per ear, the number of kernels per row, and the mean weight of 1 kernel (derived from the weight of 1000 kernels). Values for nitrogen partial factor productivity (PFP_N) of maize were also calculated for each experimental unit as the ratio between grain yield and nitrogen application rate [23], i.e., 160 and 80 kg N ha^{-1} for CF and AF, respectively.

2.4. Statistical Analysis

First, the normal distribution of all data was confirmed with a Shapiro–Wilk test [24], while homoscedasticity was validated by performing Levene's test [25]. Subsequently, all data were subjected to a three-way analysis of variance (ANOVA) with years, fertilization practices, and weed control treatments as fixed effects and replications as random effects. Because the effects of years on the parameters studied were not significant ($p \geq 0.05$), the data were pooled across growing seasons and analyzed again by a two-way analysis

(ANOVA) using the same classification of fixed and random effects. All data analyses were performed at a significance level of a = 0.05. Means were then compared using Fischer's Least Significant Difference (LSD) procedure. Statgraphics Centurion XVI (Statgraphics Technologies, Inc., P.O. Box 134, The Plains, VA, USA) was the statistical package used.

3. Results

Fertilization practices (F) affected weed density ($p \leq 0.001$), weed biomass ($p \leq 0.01$), maize grain yield ($p \leq 0.001$), and maize PFP_N ($p \leq 0.001$). The effects of weed control methods (WC) on all parameters were significant ($p \leq 0.001$). In addition, significant interactions between fertilization and weed control (F × WC) were observed for weed density ($p \leq 0.001$), weed biomass ($p \leq 0.01$), grain yield ($p \leq 0.01$), and PFP_N ($p \leq 0.001$). In contrast, years (Y) and their interactions with fertilization methods (Y × F) and weed control methods (Y × WC) had no effect on weed density, weed biomass, grain yield, and PFP_N ($p \geq 0.05$). The same ($p \geq 0.05$) was observed for the three-way interaction (Y × F × WC) between all factors (Table 2).

Table 2. The effects of years (Y), fertilization practices (F), weed control methods (WC), and their interaction (Y × F, Y × WC, F × WC, Y × F × WC) on weed density, weed biomass, maize grain yield, and maize nitrogen partial factor productivity (PFP_N). *p*-Values (*p*) are presented for each parameter as the result of a two-way analysis of variance (ANOVA) conducted at a significance level of a = 0.05. Abbreviations: B; Block, DF; Degrees of Freedom.

Factor	*Df*	Weed Density	Weed Biomass	Grain Yield	PFP_N
Y	1	0.8629	0.8598	0.6701	0.6382
B	3	0.2541	0.5481	0.9856	0.5396
Error (a) [1]	3				
F	1	0.0008	0.0012	0.0001	0.0000
Y × F	1	0.7066	0.9609	0.9960	0.9237
Error (b) [2]	6				
WC	3	0.0000	0.0000	0.0000	0.0000
Y × WC	3	0.6035	0.9393	0.8199	0.7602
F × WC	3	0.0005	0.0017	0.0038	0.0000
Y × F × WC	3	0.8378	0.9004	0.8984	0.8210
Error (c) [3]	36				
Total	63				

[1] B × Y. [2] B × F (Y). [3] B × WC (F) × Y.

Therefore, data were pooled across the two growing seasons and reanalyzed by a two-way ANOVA (Table 3).

Table 3. The effects of fertilization practices (F), weed control methods (WC), and their interaction (F × WC) on weed density, weed biomass, maize grain yield, and maize nitrogen partial factor productivity (PFP_N). *p*-Values (*p*) are presented for each parameter as the result of a two-way analysis of variance (ANOVA) conducted at a significance level of a = 0.05. Abbreviations: B; Block, DF; Degrees of Freedom.

Factor	DF	Weed Density	Weed Biomass	Grain Yield	PFP_N
F	1	0.0151	0.0255	0.0057	0.0000
B	3	0.8251	0.8379	0.9985	0.9209
Error (a) [1]	3				
WC	3	0.0000	0.0000	0.0000	0.0000
F × WC	3	0.0124	0.0157	0.0427	0.0000
Error (b) [2]	18				
Total	31				

[1] B × F. [2] B × WC (F).

Combined over the years, fertilization influenced weed density and biomass ($p \leq 0.05$), grain yield ($p \leq 0.01$), and PFP_N ($p \leq 0.001$). The effects of weed control and its interaction with fertilization (F × WC) on all studied parameters were also significant ($p \leq 0.05$).

3.1. Weed Density and Biomass

The AF resulted in 38% lower weed density compared to the CF (Figure 1a). One inter-row mechanical cultivation (M1) resulted in poor weed control and reduced weed density by approximately 50% compared to the untreated control (CON). Two cultivations reduced weed density by 57 and 78% compared to M1 and CON, respectively. Herbicide application was the most effective weed control method, reducing weed density below the 10 plants m^{-2} limit (Figure 1b). Combining the alternative fertilizer application with two mechanical passes between rows (AF × M2) or herbicide application (AF × H) resulted in the lowest weed density. In addition, AF × M2 was not significantly different from the combination of alternative fertilization and herbicide application (AF × H). AF × M1 was more effective than CF × M1 (Figure 1c).

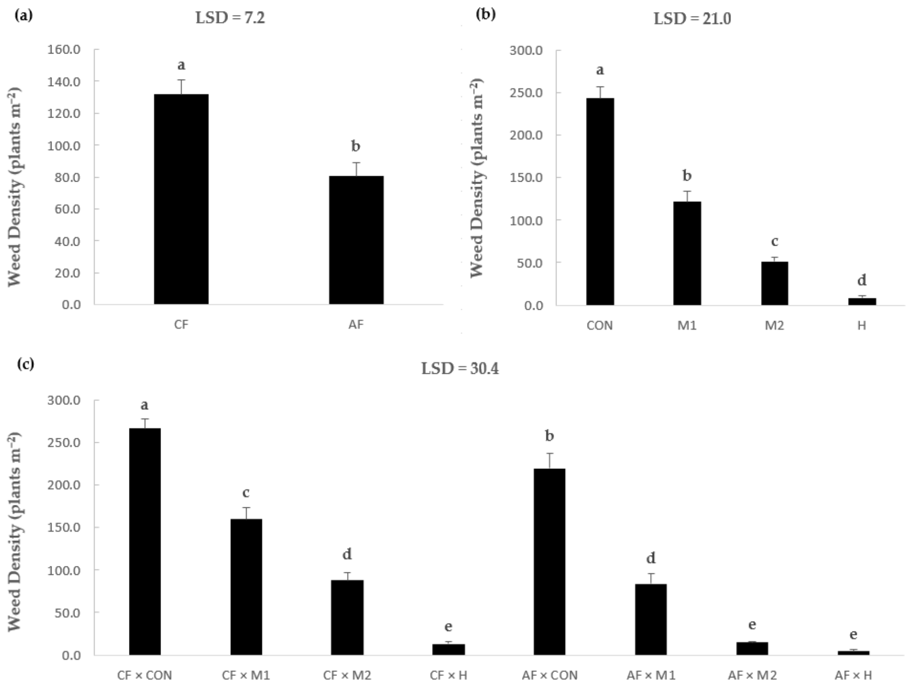

Figure 1. Weed density (plants m^{-2}) as influenced by (**a**) fertilization practices, (**b**) weed control methods, and (**c**) their interactions. Different letters indicate significant differences between means using Fischer's Least Significant Difference (LSD) procedure. Vertical bars indicate standard errors.

The weed biomass was 28% lower in the AF main plots than in the CF main plots (Figure 2a). M2 and H caused a reduction of more than 70% compared to a single mechanical pass between rows (M1). Tembotrione (H) reduced weed biomass by 53% compared to M2. Weed biomass decreased in the subplots of M1 compared to CON but still had a very high value (389 g m^{-2}; Figure 2b). The dry weight of weeds per unit area dropped below 35 g m^{-2} in AF × H and AF × M2 subplots. These interactions had values close to those of CF × H. When no weed control was applied, weed biomass exceeded 600 g m^{-2} for both fertilization treatments (CF × CON and AF × CON). The remaining interactions differed significantly in descending order: CF × M2 > AF × M1 > CF × M1 (Figure 2c).

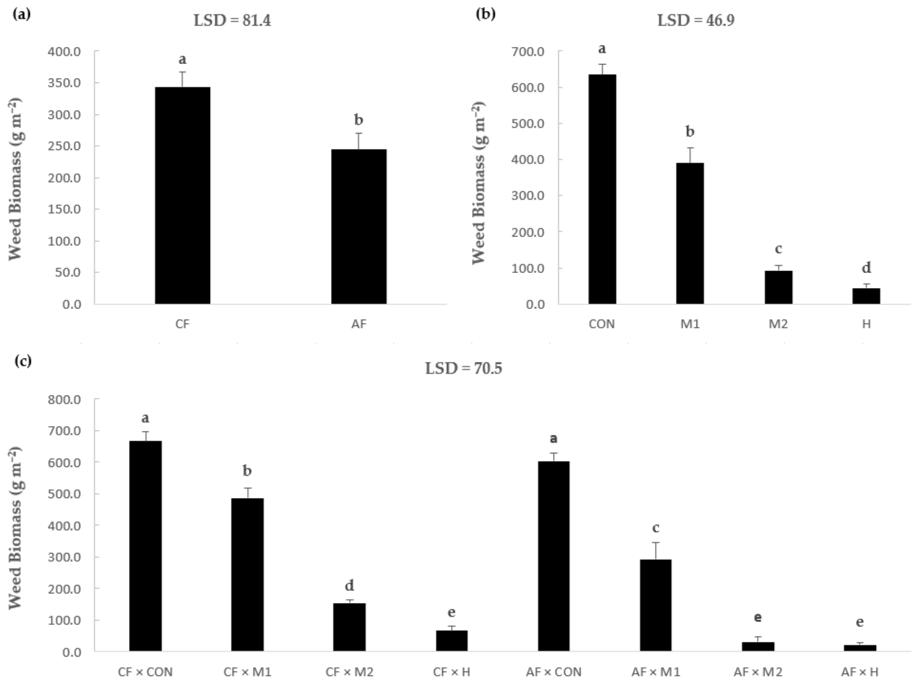

Figure 2. Weed biomass (g m^{-2}) as influenced by (**a**) fertilization practices, (**b**) weed control methods, and (**c**) their interactions. Different letters indicate significant differences between means using Fischer's Least Significant Difference (LSD) procedure. Vertical bars indicate standard errors.

3.2. Maize Grain Yield and Nitrogen Partial Factor Productivity (PFP_N)

The AF resulted in a 12% higher maize grain yield than the CF (Figure 3a). M2 increased grain yield by 18 and 25% compared to M1 and Con, respectively. M2 did not differ in any significant way from H. M1 increased yield by only 7% compared to CON (Figure 3b).

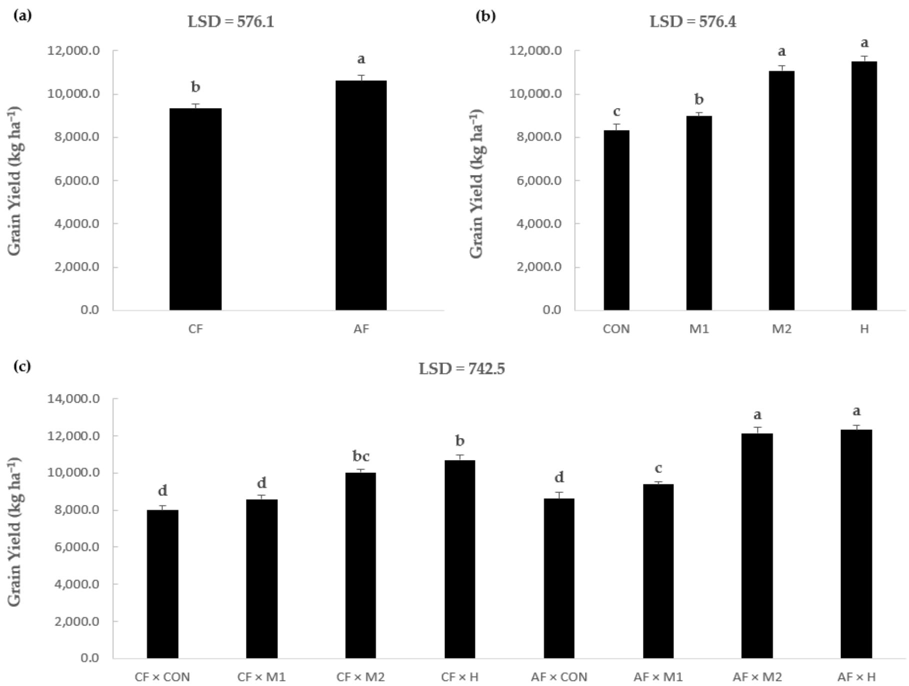

Figure 3. Maize grain yield (kg ha^{-1}) as influenced by (**a**) fertilization practices, (**b**) weed control methods, and (**c**) their interactions. Different letters indicate significant differences between means using Fischer's Least Significant Difference (LSD) procedure. Vertical bars indicate standard errors.

AF × H and AF × M2 were the highest-yielding interactions, resulting in the production of more than 12,000 kg grain ha^{-1}. These interactions increased crop yield by 10–11% compared to CF × H. The lowest grain yield values corresponded to CF × CON, AF × CON, and CF × M1. Intermediate values were observed for CF × M2 (10,021 kg ha^{-1}) and CF × M1 (9377 kg ha^{-1}), as shown in Figure 3c.

In addition, the AF improved the PFP_N of maize by 56% compared to the CF (Figure 4a). The highest values of the index were obtained in both the double inter-row mechanical weed control operation (M2) and the application of tembotrione (H). No significant differences were observed between these two weed control treatments. M1 resulted in a higher PFP_N compared to the untreated control (CON) but a significantly lower PFP_N compared to M2 and H (Figure 4b).

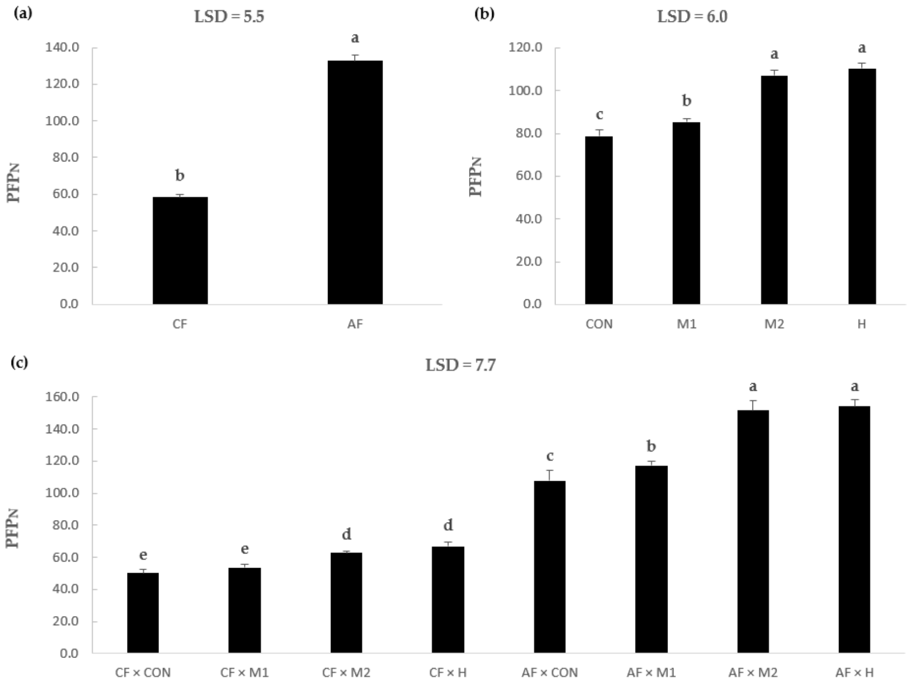

Figure 4. Maize nitrogen partial factor productivity (PFP_N) as influenced by (**a**) fertilization practices, (**b**) weed control methods, and (**c**) their interactions. Different letters indicate significant differences between means using Fischer's Least Significant Difference (LSD) procedure. Vertical bars indicate standard errors.

Regarding the effects of the interaction of fertilization practice and weed control method (F × WC) on the index, AF × M2 increased PFP_N by 56, 58, 64, and 67% compared to CF × H, CF × M2, CF × M1, and CF × CON, respectively. The alternative fertilization based on the biostimulant together with the application of the selective herbicide tembotrione (AF × H) resulted in similar PFP_N values as AF × M2. AF × CON and AF × M1 yielded a significantly higher PFP_N than CF × H, CF × M2, CF × M1, and CF × CON. In addition, subplots CF × H and CF × M2 were found to have a higher PFP_N than CF × M1 and CF × CON. AF × M2 and AF × H increased PFP_N by more than 22–30% compared to AF × M1 and AF × CON. CF × M2 and CF × H also resulted in a higher PFP_N than CF × M1 and CF × CON. These latter two interactions yielded the lowest values of the index and did not differ from each other. To summarize the differences between the interactions, they can be given in the following descending order: AF × H and AF × M2 > AF × M1 > AF × CON > CF × H and CF × M2 > CF × M1 and CF × CON (Figure 4c).

4. Discussion

The AF resulted in a lower weed density and biomass compared to the CF. This may be attributed to the lower nitrogen fertilizer input available to weeds in the AF main plots, resulting in a lower weed emergence and growth. These results agree with those of Gholamhoseini et al. [26], who also observed a 41% increase in weed biomass in their two-year field experiments with maize by increasing the nitrogen application rate from 300 to 450 kg N ha^{-1}. In another study with maize, weed density and weed biomass were consistently higher in plots receiving high nitrogen application rates with conventional broadcast fertilization than with alternative fertilization practices that reduced nitrogen levels in the soil profile [27]. Similar results were also reported by Anderson [28] for the same crop. These results are consistent with the recommendations of Di Tomaso [29] that the manipulation of fertilization strategies is one of the most important tools for weed management in field crops.

However, this study is one of the first to investigate a biostimulant containing N_2-fixing bacteria as part of a beneficial fertilization program for maize that results in a lower weed pressure. Another explanation for the current results lies in the fact that the foliar application of NitroStim® may have significantly favored crop growth and, thus, increased its competitiveness against weeds. This explanation is supported by the results of recent research by Dahiya et al. [30]. The aforementioned researchers found that the incorporation of the bacteria of the genus *Bacillus* spp. into wheat seeds resulted in up to a 76% higher shoot weight of the crop compared to the corresponding values obtained for the weed species in their study (*Avena fatua* L.).

The excellent weed control and higher yields obtained by combining biostimulants and herbicides confirm the statements of Kanatas et al. [31], who also emphasized the potential of such combinations to reduce weed infestation and increase crop yields. In the current study, a lower weed density and biomass at the time of treatment could explain the better performance of tembotrione in AF × H than in CF × H subplots. When weed density is not extremely high, it is more likely that the herbicide will be deposited optimally on the leaf surface of most weeds, resulting in an adequate coverage of their foliage and an excellent weed control [32]. On the other hand, herbicide deposition may be lower on smaller understory weed seedlings, negatively affecting herbicide efficacy in high-density plots [32]. Regarding the effects of mechanical weed control, when combined with the AF, two mechanical inter-row treatments were required to achieve a satisfactory control level. These results are consistent with other studies on legumes, small-grain cereals, and field-grown leafy vegetables whose results showed that weed density and biomass decreased with the increasing number of mechanical weed control treatments when combined with other weed-suppressive cultural practices [33–36].

Double mechanical operations usually result in greater weed control than a single treatment. This is because a single treatment eliminates the first cohorts of weeds that emerge in the field, while weeds that emerge later usually grow uncontrolled [33]. The reason that AF × M2 achieved greater weed control, and, thus, better grain yield and PFP_N, than CF × M2, could be due to the 50% higher soil nitrogen content in the CF × M2 subplots. The higher soil nitrogen levels in these plots may have promoted the emergence of post-treatment weed cohorts that escaped the second pass and competed with the crop, limiting its yield potential, as in previous studies [35]. In any case, the combination of double mechanical treatments and biostimulant application provides important benefits such as the ability to reduce herbicide and fertilizer use while improving crop yield and nitrogen utilization.

Overall, the AF significantly increased maize grain yield; the positive effects of biostimulant application on maize grain yield are well established, given the results of studies conducted under different soil and climatic conditions [37–39]. PFP_N, the ratio of grain yield to the amount of nitrogen applied, which is a useful and easily interpreted measure of nitrogen use efficiency in the cropping system, increased significantly in the AF main plots compared to the CF main plots. This is explained by the fact that the AF increased grain yield or at least maintained high values while reducing nitrogen application rates. These results are consistent with those of other researchers who also found a strong and negative relationship between PFP_N and nitrogen application rates, i.e., PFP_N increased when nitrogen application rates decreased [40–42].

However, maintaining high PFP_N levels is not the only objective of importance to farmers under real field conditions. If not accompanied by other cultural practices, a reduction in nitrogen input may well reduce the grain yield and economic benefits of a given agricultural holding [43]. Our results indicate that the application of biostimulants containing N_2-fixing bacteria could be one of the cultural practices that offset the potential negative effects on crop yields due to reduced nitrogen fertilization rates. This is because these bacteria are capable of penetrating into the aboveground plant parts (phyllosphere) and become endofytes. These nitrogen-fixing endofytes of the phyllosphere fix atmospheric nitrogen and convert it into a form that is easily assimilated by plants, ensuring a fast and balanced growth [44]. An example of other studies in which reduced nitrogen fertilization rates were combined with other cultural practices can be found in the recent study by Du et al. [45] on maize. These authors reported that increasing crop density by 30% in combination with a 15% lower base nitrogen fertilizer rate increased grain yield by more than 6%, while PFP_N increased by about 25%. Similar results have been reported for rice, cotton, and peanut [46–49].

5. Conclusions

The present study shows that plant biostimulants based on N_2-fixing bacteria enable crops to fix atmospheric N_2 and convert it to NH_3, thus providing an effective and safe means of reducing nitrogen application rates during basal dressing. As a result, weed infestation in the field is limited, and higher yields can be achieved with improved nitrogen utilization. In maize, such alternative fertilization practices become even more beneficial when combined with two mechanical inter-row cultivations or the application of a selective herbicide that further eliminates weed presence. Further studies are needed to investigate the role of biostimulants as part of integrated crop and weed management with field trials under different soil and climatic conditions. More specifically, research should focus on the potential of using biostimulants as a cultural practice that can reduce the use of fertilizers in agriculture, improve the performance of non-chemical weed control methods and herbicides, while ensuring high crop yields and effective resource utilization.

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