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Corn Response to an Integrated Plant Nutrition System (IPNS) With Humic Acid and Biofertilizers

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Abstract

Sustainable agriculture production depends on the development of methods that optimize nutrient cycling, minimize use of external inputs, and maximize input use efficiency according to the conditions of each region. The principle of an integrated plant nutrition system (IPNS) is to tailor plant nutrition and soil fertility management, taking advantage of the combined and harmonious use of inorganic, organic and biological resources. This greenhouse study investigated the individual and combined use of inorganic, organic and biological fertilizer resources for corn (*Zea mays* L.). We evaluated the effects of commercial synthetic fertilizer, humic acid products, compost/manure teas and bioinoculant as inorganic, organic and biological resources, respectively, and their synergy on corn growth and soil respiration parameters under a period of water stress. The pots were laid out in completely randomized design and the total of sixteen treatment combinations were replicated four times. In general, when comparing to the control values, the use of humic acid (HA), biofertilizers and the integration of both compounds generated significantly greater early season plant height, chlorophyll content, photosynthetic efficiency and shoot/root dry biomass. The soil substrate induced respiration was affected by only one biofertilizer product at two different rates. Though all pots received adequate synthetic fertilizer, the control plants were generally smaller and less vigorous compared to the plants receiving either HA or biofertilizer treatments, but no additive benefit was observed for the integrated practice compared to individual applications. Further studies addressing different types and levels of stress along with greater stress duration should be conducted to validate these findings.

Keywords: bioinoculants, compost tea, manure tea, organic fertilizer, humates, water stress, plant biostimulants

1. Introduction

Successful crop production relies on nutrients that are available in sufficient quantities and forms to promote satisfactory plant growth. Fertilization is an essential practice to enhance soil fertility, increase crop productivity and support agricultural intensification (Vaneeckhaute et al., 2013). Optimized fertilization schemes require methods to optimize nutrient cycling, minimize use of external inputs, and maximize input use efficiency appropriate to the conditions of each region (Cakmak, 2002; Kumwenda, Waddington, Snapp, Jones, & Blackie, 1996).

A huge variety of materials can serve as sources of plant nutrients. These can be inorganic, organic, recycled wastes or a range of biological products including compost teas and microbial inoculants. The nature and the characteristics of nutrient release from fertilizers derived from inorganic, organic and biological resources differ and thus must be managed differently (Chen, 2006; Dutta, Pal, Chakraborty, & Chakrabarti, 2003). Sustaining high crop yields should include not only the addition of synthetic fertilizer materials but also the integrated use of biological and organic nutrient resources as a way to increase nutrient use and minimize environmental

impacts (Hussain, Jilani, & Iqbal, 1988; Kaur, Kapoor, & Gupta, 2005). According to the Food and Agriculture Organization of the United Nations (FAO) (Shand, 2007), the definition of an IPNS is "the adaptation of the plant nutrition and soil fertility management in farming systems to site characteristics, taking advantage of the combined and harmonious use of inorganic, organic and biological nutrient resources to serve the concurrent needs of food production and economic, environmental and social viability." The principle of IPNS requires an understanding of nutrient dynamics throughout the soil-microbe-plant systems in order to regulate the availability of nutrients derived from inorganic, organic and biological sources to address long- and short-term crop production and environmental impacts (Aulakh & Grant, 2008).

Several studies addressing different crop species have shown the beneficial effects of the integrated use of different fertilizer or biostimulant sources on yield, shoot and root growth, and nutrient uptake on different species (Adesemoye, Torbert, & Kloepper, 2008; Mantelin & Touraine, 2004) such as sugarcane (*Saccharum officinarum* L.) (Bokhtiar & Sakurai, 2005; Sundara, Natarajan, & Hari, 2002) and red pepper (*Capsicum annuum* L.) (Joo, Kim, Lee, Song, & Rhee, 2004). However, in managed ecosystems, the dynamics of nutrient availability will vary depending on the nutrient resource applied.

The heterogeneous and complex molecules present in humic substances have shown many positive effects on plant growth, nutrient uptake efficiency and soil. Commonly, these effects can be intercorrelated and complementary. Plant growth stimulation from the use of HA has been reported in such ways as increased berry size and improved fruit quality in table grapes [*Vitis Vinifera* (L.) cv. Italia] (Ferrara & Brunetti, 2010) and greater root growth on Brazilian red cloak (*Megaskepasma erythrochlamys*) and sanchezia (*Sanchezia nobilis* L.) (Baldotto & Baldotto, 2014) and tobacco (*Nicotiana tabacum* L.) (Mylonas & McCants, 1980). Humic compounds stimulate plant development through improving nutrient adsorption on lily (*Lilium* L.) (Chang, Wu, Xu, Nikbakht, & Xia, 2012) and gerbera (*Gerbera jamesonii* L.) (Nikbakht et al., 2008). Furthermore, humic acids can promote vegetal growth by mediating biochemical, morphological, physiological processes (Chen, Senesi, & Schnitzer, 1977; Tahiri, Destain, Thonart, & Druart, 2015; Vaughan & Malcolm, 1985).

The application of HA can positively affect soil cation exchange capacity and nutrient availability which indicates that HA materials may serve as resources that can improve fertilization efficiency (Albiach, Canet, Pomares, & Ingelmo, 2001; Tahiri et al., 2015; Vaughan & MacDonald, 1976). Thus, the physicochemical activity and structure of HA substances might increase agriculture production through improved soil quality and by enhancing soil stability and resistance to erosion (Brannon & Sommers, 1985; Spaccini, Piccolo, Conte, Haberhauer, & Gerzabek, 2002). The dual beneficial effects of HA on soil and plant might explain the production increase on tomato (*Solanum lycopersicum* L.), cotton (*Gossypium arboretum* L.) and grapes (*Vitis vinifera* L.) (Brownell, Nordstrom, Marihart, & Jorgensen, 1987) as improved soil promotes better conditions for plant growth.

Several studies have documented enhancement of vegetative growth, yield and nutrient uptake by improving the physico-chemical properties of the soil (Kim et al., 2015; Siddiqui, Islam, Naidu, & Meon, 2011) and incremental benefits to the microbial population for plants and soil fertility (Chen, 2015) in response to compost tea (CT) application. Moreover, solubilization of P or K, uptake of N and multiplication of extraradical hyphae biomass are effects promoted by biofertilizers that might minimize negative impacts of soil degradation, in addition to induction of plant growth (Bianciotto & Bonfante, 2002; Rodríguez & Fraga, 1999). Therefore, the application of microbial inoculants has shown the potential to improve sustainable production in intensive agriculture systems (Bhattacharyya & Jha, 2012; Chauhan, Bagyaraj, Selvakumar, & Sundaram, 2015) due to nutrient release, plant growth stimulation, rhizoremediation and plant stress control (Lugtenberg & Kamilova, 2009).

In fact, the potential benefits of HA and biofertilizers as plant-growth promoters for increased nutrient acquisition (Yildirim, 2007), increased stress tolerance (Zhang & Ervin, 2004), and pathogen suppression (On et al., 2015) are evident in the literature, and substantial work has been done in this area. Thus, plant–microbial symbioses are very important components of nutrient cycling in agroecosystems and enhance plant nutrient uptake (Peoples & Craswell, 1992; Zhu, Cavagnaro, Smith, & Dickson, 2001). Studies have shown that HA stimulates microbial effects on ion exchange and metal complexing (chelating) systems (Puglisi et al., 2009; Visser, 1985). Also, HA has increased the production of micelium by mycorrhizal fungi (Gryndler et al., 2005) and promoted greater nodule formation of native rhizobia (Gaur & Bhardwaj, 1971).

Despite the recognition of the independent effects of HA and biofertilizers, few applicable studies have been conducted to elucidate the interaction of HA and biofertilizers on agronomic, economic and/or environmental outcomes. Moreover, there is a lack of knowledge regarding the effects of HA on plant–microbial symbioses.

Therefore, the present study evaluated the effects of the combined and individual use of HA and compost/manure teas and bioinoculants along with inorganic fertilizer on corn (*Zea mays* L.) development and soil respiration. We hypothesize that the synergetic effects of the combination of HA + biofertilizer will improve corn agronomic outcomes and increase soil respiration when comparing the application of each product alone.

2. Materials and Methods

In this greenhouse study, humic acid (HA) was used as an organic resource and compost/manure tea and bioinoculants were used as biological resources along with conventional inorganic fertilizer resources (NPK) in an integrated manner.

2.1 Products Description (Treatments)

Seven products, including inorganic, organic and biological resources, were used in this study. The inorganic fertilizer was Osmocote Plus®; the organic product was MicroLife Humic Acid Complex®; and the biological products were SoilSoup®, Microgeo® and Microgro Supreme Bioinoculant®. The MicroLife 6-2-4® and Nanobind® are derived from organic and biological resources. The Osmocote Plus® is a slow release synthetic fertilizer containing 11 essential nutrients for plants. The organic/ humic category was represented by MicroLife Humic Acid Complex® which was constituted of 15% humic acid and 1% fulvic acid. One of the three biological fertilizers was Microgeo®, which is a Brazilian patented product categorized as a manure tea. This biofertilizer is composed of organic compounds, active and dormant cells from various microorganisms (bacteria, yeasts, filamentous fungi, and algae), metabolites and organo-mineral chelates and it is produced through continuous anaerobic fermentation in a liquid media (D'Andrea, 2002). According to the technical manual, the preparation is using the CLC® (Continuous Liquid Composting) process, where 5% of the commercial biological fertilizer Microgeo ®, 15% of ruminal content and water are mixed in a tank exposed to sunlight. After 15 days the biofertilizer is ready to be applied. SoilSoup® is an aerobic compost tea generated via fermentation of vermicompost over 24 hours with the addition of nutrient solution (molasses, bat guano, sea bird guano, soluble kelp, langbeinite, natural citric acid, ancient seabed minerals, yucca) and oxygen to the system (aquarium pump). The Microgro Supreme Bioinoculant® is a water-soluble powder containing 76 different strains of bacteria and fungi including 11 different Mycorrhizal species and microbial food (sugars, humic acid, kelp, amino acids and yeast extract). The MicroLife 6-2-4® is a pelletized fertilizer that contains 6, 2 and 4% N, P and K, respectively. These nutrients are derived from a combination of organic and biological materials including fish, kelp, molasses, humates, bat guano, rock phosphate, wheat middlings, soy meal, cottonseed meal, alfalfa, corn meal, potassium sulfate, iron sulfate, Folic Acid, vitamins and bioinoculants. Nanobind® is constituted by the combination of humic substances and microbial inoculants. The products' descriptions are summarized in Table 1.

Table 1. Product description

Resource	Category	Subcategory	Name	Components
Inorganic	Synthetic		Osmocote Plus	Polymer-coated: Ammonium Nitrate, Ammonium Phosphate, Potassium Sulfate, Magnesium Sulfate, Sodium Borate, Iron Phosphate, Iron EOTA, Manganese Sulfate, Sodium Molybdate, Aibc Sulfate, Copper Sulfate and Zinc Oxide.
Organic	Humic	Fulvic	Microlife Humic Acid Complex	15% Humic Acid and 1% Fulvic Acid derived from leonardite
Organic + Biological	Biofertilizer	Manure tea	Microgeo	Recancitran Substances, Biodynamic Preparations, Pentoses, Minerals and Brans and the microorganisms produced in the manure tea fermentation
		Compost tea	SoilSoup	Molasses, Bat Guano, Sea Bird Guano, Soluble Kelp, Langbeinite, Natural Citric Acid, Ancient Seabed Minerals, Yucca and the microorganisms produced in the compost tea fermentation
		Bioinoculant	Microgro Supreme Bioinoculant	76 different strains of bacterias and fungi planced on dry milk carrier loaded with microbial food. The microorganisms included are: species of Genus Bacillus, Psuedomonas,Streptomycetes, Trichoderma, and Endo and Ectomycorrhizal Fungi
			Microlife 6-2-4	Fish, Kelp, Molasses, Emery Humates, Bat Guano, Rock Phosphate,Wheat Middling's, Soy Meal, Cottonseed Meal,Alfalfa, Corn Meal, Potassium Sulfate, Iron Sulfate, Amino Acids, Folic Acid,Vitamins and MicroGro Supreme BioInoculant
			Nanobind	Lactobacillus culture, Saccharomyces Boulardii culture, Phytase enzymes, Lipase enzymes, Amylase enzymes, Superoxide Dismutase enzymes, Protease enzymes, organic carbon (humic)

2.2 Experimental Design and Management

The experiment was conducted under controlled conditions in a greenhouse in Blacksburg (Virginia, USA) to investigate the individual and combined effects of humic acid (HA), compost/manure tea and bioinoculants on corn growth. Polyethylene pots (19 cm tall, 19 cm outside diameter, and 37851 cm^3 volume) were lined with plastic bags to avoid water loss. Soil media and sand (50% Metro-mix 360 and 50% playground sand, respectively) were placed in a polyethylene pot and 21 g of inorganic fertilizer (Osmocote Plus®) was equally added in each pot. According to the bulk density provided in the physical/chemical characteristics data sheet of each component, we added 0.425 kg Metro-mix 360 and 3 kg sand to each pot to have an equal volume. Posteriorly, corn seeds were planted by hand at 3 cm depth and thinned to one seedling after germination.

The field capacity on the soil media + sand was determined after water saturation until the first drop of water leached through the bottom of the pot. Then, after 1 day the weight of the pot containing the wet soil was taken to be used as field capacity threshold (Kirkham, 2014).

We employed 6 treatments, each at two concentration levels, 1x and 2x the label rate of each product, depending on the treatment (Table 2). The trial used a completely randomized design (CRD) with four replications. Each treatment was applied at corn growth stages V1, V4, V6 and V8. The treatments were previously prepared in the laboratory and applied into each pot using an electronic pipette. Solid materials were dissolved in water and the appropriate rate applied to respective pots.

Table 2. Treatments and application rate

No.	**Treatments**			
	Product Name and Abbreviation	Rate	Label	Rate/pot (each application)
1.	Microgeo (M)	1x	150 l/ha	0.47 ml
2.	Microgeo (M)	2x	150 l/ha	0.94 ml
3.	Soil Soup (S)	1x	235 l/ha	0.73 ml
4.	Soil Soup (S)	2x	235 l/ha	1.46 ml
5.	Microgro Supreme Bio inoculant (MB)	1x	6.1 kg/ha	19 mg
6.	Microgro Supreme Bio inoculant (MB)	2x	6.1 kg/ha	38 mg
7.	Microlife Humic (H)	1x	14 l/ha	0.043 ml
8.	Microlife Humic (H)	2x	14 l/ha	0.086 ml
9.	Nanobind (N)	1x	4.6 l/ha	0.015 ml
10.	Nanobind (N)	2x	4.6 l/ha	0.030 ml
11.	Microlife 6-2-4(ML)	1x	975 kg/ha	3000 mg
12.	Microlife 6-2-4(ML)	2x	975 kg/ha	6000 mg
13.	Microgeo + Microlife Humic (M + H)	1x, 1x	150 l/ha and 14 l/ha	0.47 ml + 0.043 ml
14.	Soil Soup + Microlife Humic (S + H)	1x, 1x	235 l/ha and 14 l/ha	0.73 ml + 0.043 ml
15.	Microgro Supreme Bio inoculant + Microlife Humic (MB + H)	1x, 1x	6.1 kg/ha and 14 l/ha	19 mg + 0.043 ml
16.	Control (C)	0x	0	0

Note. The surface area on top of the pot was 314 cm^2.

2.3 Water Regime and Data Collection

The pots were maintained at 60% of field capacity for the first 40 days of the experiment to ensure adequate moisture for corn growth. Between 40 and 50 days post-emergence (PE), watering was reduced to 30% of field capacity to induce mild to moderate drought stress. Plant height at the leaves within the whorl, atLEAF chlorophyll meter value (FT Green LLC, Wilmington, DE) and photosynthetic efficiency/OS-50II fluorometer (Opti-Sciences, Tyngsboro, MA) measurements were collected from the latest fully developed leaf defined using the leaf collar method (Abendroth, Elmore, Boyer, & Marlay, 2011) at 20, 40 and 60 days PE. At 60 days post-emergence, the aboveground plant material was clipped at the soil surface and dried at 70 °C until a constant weight was achieved so that plant dry matter yield could be calculated. Corn growth stages corresponding to 20, 40 and 60 days post-emergence were V4, V6 and V8, respectively. After aboveground biomass harvest, roots were separated from the soil media + sand by shaking and root dry matter calculated in a similar manner to the shoot.

2.4 Substrate-Induced Respiration

A subsample of soil from the whole pot (300 g) was collected at the end of the growth period following aboveground and root biomass collection. Substrate-induced respiration (SIR) was performed to determine active microbial biomass in soil samples (Fierer, Schimel, & Holden, 2003). The collected samples were weighed (4g dry weight equivalent) into modified 250 ml centrifuge tubes modified with holes drilled in the tube caps and filled with rubber caulk to facilitate gas extraction. Soils were conditioned to an incubation temperature of 20°C prior to the addition of substrate. To each sample, 8 ml of yeast substrate was added (12 g BD Bacto™ yeast extract/liter H_2O) and the sample was placed on a shaker for 1 hour. After thoroughly mixing substrate and soil, the tubes were tightly sealed and flushed with CO_2 free air for 3 minutes. After incubation at 20°C for 5 hours, a syringe was used to remove 5 ml of headspace gas from the sealed tubes. Analysis of the sample was performed with a Licor model LI-7000 infrared gas analysis (IRGA) (LI-COR Corporate, Lincoln, NE) to determine CO_2 concentration and soil respiration rate (ug CO_2/g dry soil/hour).

2.5 Data Analysis

Analysis of variance using PROC GLM of SAS 9.4 (SAS Institute, 2011) was conducted to evaluate treatment effects on plant height, atLEAF chlorophyll meter values, photosynthetic efficiency and root, shoot and total biomass. Differences between treatments and control means were separated using Dunnett's test and the t-test of the means were deemed significant differences when F-test values were $\alpha < 0.05$ for the plant parameters and $\alpha < 0.1$ for the SIR. Single-degree of freedom contrasts were used to determine significant differences between rates of the same product.

3. Results and Discussion

3.1 Plant Height

Generally, treatments positively impacted plant height to a greater degree as the study progressed from 20 to 60 days PE (Table 3).

Table 3. Analysis of variance of the effects of IPNS treatments on plant height, atLEAF and Fluoremeter at 20, 40 and 60 days post-emergence (PE)

Source	Plant height			atLEAF			Fluorometer		
	20 days PE	40 days PE	60 days PE	20 days PE	40 days PE	60 days PE	20 days PE	40 days PE	60 days PE
	Pr > f								
Rep	0.2985	0.0047	<0.0001	0.1869	0.1316	0.0095	<0.0001	0.0113	0.5537
Treatment	0.044	0.0002	<0.0001	0.0089	0.1715	0.0265	<0.0001	<0.0001	<0.0001
CV	13.8	7.0	8.2	7.9	7.0	3.1	4.8	3.3	2.4
SED	6.1	4.2	10.3	4.5	3.8	2.0	0.034	0.026	0.019

Progressively, the number of treatments with plant height significantly greater than the control increased as the study progressed. The number of treatments with plants significantly taller than the control increased from 5 to 7 and then 11 at 20, 40 and 60 days PE, respectively (Table 4). Mbagwu and Piccolo (1997) tested the responses of coal-derived humic substances on corn and they also found increases in plant height, even though the plants in their research were generally shorter than this current study when comparing the plant height around 5 weeks after emergence. Several studies have shown the influence of biofertilizers on plant height stimulation of species like potato (*Solanum tuberosum* L.), tomato, maize (Bhattacharyya & Jha, 2012), tobacco (Zhang & Kong, 2014) and wheat (*Triticum aestivum* L.) (Aftab & Asghari, 2008). Moreover, a study on compost tea reported a pattern of increased plant height of lettuce (*Lactuca sativa* L.), soybean (*Glycine max* L.) and sweet corn as concentration of compost tea increased from 0.1%, 0.2%, 0.4%, to 0.8% of the total application (Kim et al., 2015).

Table 4. Mean height of control plants and differences between height of treatment and control at 20, 40 and 60 days PE

Category	Treatment comparison	Difference between treatments and control values		
		20 days PE	40 days PE	60 days PE
		---------- Plant height, cm ----------		
Biofertilizer	M-C (1x)	13.0 *	11.8 *	28.0 *
	M-C (2x)	14.9 *	11.8 *	31.5 *
	S-C (1x)	9.1	10.0 *	32.0 *
	S-C (2x)	12.1	14.8 *	37.2 *
	MB-C (1x)	11.1	14.3 *	32.6 *
	MB-C (2x)	10.8	10.8 *	36.2 *
Humic	H-C (1x)	8.9	7.0	30.6 *
	H-C (2x)	13 *	8.3	21.0
Humic + Biofertilizer	N-C (1x)	11.4	4.0	3.4
	N-C (2x)	4.8	4.5	16.2
	ML-C (1x)	10.8	9.0 *	25.8 *
	ML-C (2x)	5.4	1.3	21.6 *
	M+H-C (1x,1x)	11.7	6.8	21.3
	S+H-C (1x,1x)	15.9 *	8.8	22.9 *
	MB+H-C (1x,1x)	15.6 *	8.5	21.9 *
Actual Control values (C)		33.3	52.3	102.5

Note. * denotes significant differences, $\alpha < 0.05$.

M = Microgeo; S = SoilSoup; MB = Microgro Supreme Bio Inoculant; H = Microlife Humic; N = Nanobind; ML = Microlife 6-2-4; C = Control.

Figure 1. Plant height visual contrast between control (left) and Microgeo 2x treated plants (right) at 60 days post-emergence

3.2 Chlorophyll Content (atLAEF) and Photosynthetic Efficiency (F_v/F_m)

The total photosynthetic pigments or chlorophyll content has been used to assess the physiological status of plants and to detect stress conditions such as high salt level in soil (Taïbi et al., 2016) and drought (Zhang et al., 2011). The statistical significance of atLEAF values were less drastic than that of fluorometer (Table 3).

Generally, atLEAF values did not differ between treatments and the control with some values lower than the control (Table 5). At 40 days PE, water content in the pots was dropped to 30% of field capacity and the treatments combining HA + biofertilizer had greater atLEAF values at 60 days PE in response (Table 5). This may indicate that corn plants receiving these treatments suffered less stress from water limitation. In contrast to our results where differences in indirect measures of chlorophyll content were scarce, other studies have reported that HA consistently increases chlorophyll content in potato leaf tissue (Selim, Shedeed, Asaad, & El-Neklawy, 2012) and roselle (*Hibiscus sabdariffa* L.) (Sanjari, Sirousmehr, & Fakheri, 2015) under hydric stress conditions. Azab (2016) revealed that biofertilizers alone and in combination with NPK increased chlorophyll content of corn under moderate, intermediate and severe water deficit. Also, the same study showed that biofertilizer + 50% NPK produced greater chlorophyll content than the application of biofertilizer + 100% NPK under normal irrigation and water deficit. Abdelraouf, El-Habbasha, Hozayn, and Hoballah (2013) found that the application of biofertilizer to wheat significantly increased total chlorophyll under 100%, 80%, 60% and 40% irrigation requirements compared to treatments without biofertilizer. Furthermore, to clarify the relationship between atLEAF and the actual chlorophyll content, devices that provide a non-destructive estimate of the amount of chlorophyll present in the plant leaf (Gianquinto et al., 2004) and strong relationships between these chlorophyll meters readings and the actual chlorophyll content in the leaves in many different crops (Pellizzaro, Ventura, Arca, & Canu, 1998) including corn (Castelli, Contillo, & Miceli, 1996; Markwell, Osterman, & Mitchell, 1995) have been reported. The SPAD-502 Chlorophyll meter (Soil Plant Analysis Development, Minolta Camera Co., Ltd., Japan) is the most used device, however the atLEAF Chl meter (FT Green LLC, Wilmington, DE) used in this study can be an affordable alternative to the SPAD-502 meter (Zhu, Tremblay, & Liang, 2012).

Photosynthetic efficiency values were higher than control values for most treatments in all three data collection periods (Table 6). A more energetic photosynthesis process could affect plant development such as greater plant height and biomass values measured in this study. According to Björkman and Demmig (1987) the optimal value of F_v/F_m is around 0.83 for most species, depending on the developmental stage of the leaves, with lower values indicating plant stress. Thus, the photosynthetic efficiency readings collected in the most mature period (60 days PE) showed that the treatments presenting significant differences between control were much closer to the optimal/non-stress threshold. Lotfi et al. (2018) tested the effects of HA on photosynthetic efficiency of rapeseed (*Brassica napus* subsp. *napus*) plants in different water regimes and the application of HA resulted in increased maximum quantum yield of PSII photochemistry (F_v/F_m), where the highest discrepancy between non-humic acid and HA treatments appeared in response to the most severe water stress. Shool and Shamshiri (2014) tested the interaction effect of mycorrhizal fungi *Glomus mosseae* and the bacterial strain *Pseudomonas fluorescens* P_{52} in pistachio (*Pistacia vera*) cv. Qazvini plants under water regimes of 100%, 75%, 50% and 25% of field capacity and the highest discrepancy of F_v/F_m values between non-biological and biological fertilization appeared in the treatments managed under 25% of field capacity.

The chlorophyll works as a photoreceptor in photosynthesis, thus there are studies showing the correlation between total chlorophyll content and F_v/F_m in aloe vera (*Aloe vera*) (Hazrati, Tahmasebi-Sarvestani, Modarres-Sanavy, Mokhtassi-Bidgoli, & Nicola, 2016), olive tree (*Olea europaea*) (Khaleghi, Arzani, Moallemi, & Barzegar, 2012) and wheat (Sharma, Andersen, Ottosen, & Rosenqvist, 2015). These studies showed lower values of total chlorophyll and F_v/F_m during water or heat stress and higher values when the plants were experiencing ideal conditions. When comparing these previous studies with this current study, we found similar relationships for chlorophyll content and F_v/F_m. However, the chlorophyll content and F_v/F_m relationships in our study were not as evident as the values presented in the three studies mentioned before. In fact, the difference between control and treatments was more evident in the F_v/F_m than atLEAF / chlorophyll content readings (Tables 5 and 6).

Table 5. atLEAF readings represented by the actual control values and the difference between treatment and control values

Category	Treatment comparison	Difference between treatments and control values		
		20 days PE	40 days PE	60 days PE
		atLEAF, unit		
Biofertilizer	M-C (1x)	3.63	7.73	2.08
	M-C (2x)	2.95	5.55	1.93
	S-C (1x)	5.10	7.20	3.53
	S-C (2x)	4.15	6.70	1.33
	MB-C (1x)	3.85	7.92 *	3.03
	MB-C (2x)	5.08	5.33	3.93
Humic	H-C (1x)	7.28	5.30	4.15
	H-C (2x)	-0.03	6.88	3.93
Humic + Biofertilizer	N-C (1x)	-2.48	3.15	2.83
	N-C (2x)	9.08	4.45	4.03
	ML-C (1x)	8.03	5.93	4.98 *
	ML-C (2x)	8.80	5.20	1.48
	M+H-C (1x,1x)	6.78	7.48	4.80 *
	S+H-C (1x,1x)	10.15 *	10.02 *	4.87 *
	MB+H-C (1x,1x)	6.20	6.98	4.30 *
Actual Control Values (C)		52.23	47.85	60.88

Note. * denotes significant differences, $\alpha < 0.05$.

M = Microgeo; S = SoilSoup; MB = Microgro Supreme Bio Inoculant; H = Microlife Humic; N = Nanobind; ML = Microlife 6-2-4; C = Control.

Table 6. OS-50II fluorometer readings (Photosynthetic efficiency) represented by the actual control values and the difference between treatment and control values

Category	Treatment comparison	Difference between treatments and control values		
		20 days PE	40 days PE	60 days PE
		Fluorometer, F_v/F_m		
Biofertilizer	M-C (1x)	0.258 *	0.112 *	0.057 *
	M-C (2x)	0.260 *	0.123 *	0.070 *
	S-C (1x)	0.125 *	0.048	0.008
	S-C (2x)	0.136 *	0.049	0.003
	MB-C (1x)	0.248 *	0.117 *	0.074 *
	MB-C (2x)	0.272 *	0.129 *	0.069 *
Humic	H-C (1x)	0.238 *	0.131 *	0.031
	H-C (2x)	0.211 *	0.106 *	0.031
Humic + Biofertilizer	N-C (1x)	0.134 *	0.101 *	0.013
	N-C (2x)	0.159 *	0.069 *	0.006
	ML-C (1x)	0.248 *	0.114 *	0.070 *
	ML-C (2x)	0.246 *	0.133 *	0.049 *
	M+H-C (1x,1x)	0.311 *	0.136 *	0.071 *
	S+H-C (1x,1x)	0.225 *	0.113 *	0.046 *
	MB+H-C (1x,1x)	0.289 *	0.130 *	0.070 *
Actual Control Values (C)		0.489	0.669	0.742

Note. * denotes significant differences, $\alpha < 0.05$.

M = Microgeo; S = SoilSoup; MB = Microgro Supreme Bio Inoculant; H = Microlife Humic; N = Nanobind; ML = Microlife 6-2-4; C = Control.

3.3 Plant Biomass

Final shoot, root and total dry biomass were all significantly affected by IPNS treatment (Table 3). The greatest effect of IPNS treatment occurred for root biomass receiving HA treatments and shoot biomass receiving the biofertilizer treatments (Table 7). Total biomass was also affected by most of the treatments, where only 3 treatments did not present significantly higher value than control. Previous studies have reported increased shoot and root biomass when HA and/or biofertilizers were applied, especially under stress conditions (Dimkpa, Weinand, & Asch, 2009; Prado et al., 2016).

Shoot and root dry weight are commonly used to measure the effects of humic substances (Chen & Aviad, 1990) and in this corn study, the HA treatments resulted in the greatest root biomass. Chen and Aviad (1990) mentioned that the increased root growth promoted by HA is generally more evident than shoot growth, which is also what we observed in the current study. Other researchers have documented increased root biomass when HA was applied in soybeans (Prado et al., 2016), lettuce (Young & Chen, 1997), bentgrass (*Agrostis palutris*) (Dorer & Peacock, 1997), forage turnips (*Brassica rapa* L.) (Albayrak & Camas, 2005) and tomato (Adani, Genevini, Zaccheo, & Zocchi, 1998). In contrast, (Hartz & Bottoms, 2010) tested five commercial HA formulas and found no significant effect on tomato dry mass accumulation. Therefore, the effectiveness of HA will depend on product rate, severity of stress, organic matter content of the soil, HA composition and extraction method. According to Tahiri et al. (2015), humic substances influence two main mechanisms of plant growth: improvement of nutrient availability and phyto-stimulation. The use of HA enhanced the adsorption of macro and micro nutrients of gerbera (Nikbakht et al., 2008) and the presence of physiologically active concentrations of cytokinin in humic substances was demonstrated in a study using radish (*Raphanus sativus* L.) and corn plants (Pizzeghello, Francioso, Ertani, Muscolo, & Nardi, 2013). Though the effects of humic substances on root biomass have solid evidence, a number of studies also present beneficial effects of HA on length and fresh and dry weight of shoots (Nardi, Carletti, Pizzeghello, & Muscolo, 2009).

Biofertilizers most often affected shoot biomass (Table 7). Previous studies have reported significant increases in shoot dry biomass for wheat (Singh & Prasad, 2011), rice (*Oryza sativa* L.) (Yuwono, Handayani, & Soedarsono, 2005) and lettuce (Kohler, Caravaca, & Roldán, 2010) when various biofertilizers were applied. Application of biofertilizers derived from vermicompost tea also outperformed the control in terms of shoot biomass on tomatoes (Edwards, Arancon, & Greytak, 2006; Fritz, Franke-Whittle, Haindl, Insam, & Braun, 2012). The plant growth effects caused by the use of biofertilizers have been attributed to increased microbial population, biologically active substances and nutrition promotion by accelerating mineralization processes (Rodríguez & Fraga, 1999; Somers, Vanderleyden, & Srinivasan, 2004). It was also postulated that the growth stimulation might be due to the phytohormones synthesizing as auxins (Dobbelaere, Croonenborghs, Thys, Broek, & Vanderleyden, 1999), gibberellic acids (Turan et al., 2014), and cytokinins (Zhang et al., 2014). Biofertilizer treatments alone affect root biomass to a much lesser extent comparing to the other materials (Table 7), however there are several studies showing the benefits of biofertilizers on root growth in several crops (Bhardwaj, Ansari, Sahoo, & Tuteja, 2014) and wheat (Dobbelaere et al., 1999).

In general, the use of HA and/or biofertilizers increased total plant biomass compared to the control, however the integrated use of these compounds interestingly resulted in plants with more proportional above/belowground biomass ratio. A lower shoot:root ratio (Table 7) could indicate greater stress tolerance at a more mature growth stage because a proportional root system may have improved ability to send nutrients/water to the aboveground biomass. In both greenhouse and field trials, Canellas et al. (2013) validated a synergistic effect of biofertilizer and HA, where corn grain yield was 45% and 48% higher with the integrated use of both compounds when comparing with the independent use of biofertilizer and HA, respectively.

Table 7. Shoot, root and total dry biomass readings represented by the actual control values and the difference between treatment and control values. Shoot-root ratio is an absolute value

Category	Treatment comparison	Difference between treatments and control values						
		Shoot biomass		Root biomass		Total biomass		Shoot-root ratio
		g						
Biofertilizer	M-C (1x)	25.25	*	4.65	*	29.90	*	3.77
	M-C (2x)	33.80	*	2.23		36.03	*	4.92
	S-C (1x)	34.75	*	1.68		36.43	*	5.17
	S-C (2x)	33.67	*	0.25		33.92	*	5.63
	MB-C (1x)	24.72	*	1.65		26.37	*	4.50
	MB-C (2x)	13.33		2.38		15.71		3.56
Humic	H-C (1x)	18.60		6.25	*	24.85	*	3.12
	H-C (2x)	11.78		7.02	*	18.80		2.66
Humic + Biofertilizer	N-C (1x)	14.13		6.60	*	20.73	*	2.84
	N-C (2x)	8.08		6.00	*	14.08		2.61
	ML-C (1x)	24.67	*	5.57	*	30.24	*	3.56
	ML-C (2x)	13.00		3.40		16.40		3.32
	M+H-C (1x,1x)	12.10		8.77	*	20.87	*	2.47
	S+H-C (1x,1x)	17.90		6.95	*	24.85	*	2.98
	MB+H-C (1x,1x)	12.28		12.27	*	24.55	*	2.13
Actual Control Values (C)		42.10		13.23		55.33		3.19

Note. * denotes significant differences, $\alpha < 0.05$.

M = Microgeo; S = SoilSoup; MB = Microgro Supreme Bio Inoculant; H = Microlife Humic; N = Nanobind; ML = Microlife 6-2-4; C = Control.

Figure 2. Root biomass visual contrast between control (left) and Microgro Supreme Bio Inoculant + Microlife Humic (right)

3.4 Differences between the Two Application Rates of the Same Product

The treatments using only one product were tested at 1x and 2x the label rate per application. The statistically significant differences between the two application rates (1x and 2x) were scarce considering the products tested and parameters collected. MicroLife 6-2-4 at the lower rate (1x) resulted in greater differences in total biomass, plant height (40 days PE) and atLeaf (60 days PE) (Table 8). Lower rates (1x) of Microlife humic produced greater atLEAF values at (20 days PE) (Table 8). The only product where doubling the rate generated significant greater difference was the application of Nanobind (2x) on atLEAF (20 days PE) (Table 8). Thus, it might be reasonable to affirm that the companies are providing a proper label rate for each product considering the two rates tested in this study. Nikbakht et al. (2008) have tested five different levels of HA and found that higher

levels decreased absorption of some nutrients, confirming the importance of suitable application rate. Furthermore, Vallini, Pera, Avio, Valdrighi, and Giovannetti (1993) found the optimal dose of humic acid + *Glomus mosseae* and the treatments where HA concentration was above the optimal dose the laurel (*Laurus nobilis* L.) shoot and root fresh weight decreased to values lower than the control in which no HA was applied.

Table 8. Plant height contrast between 1x and 2x of the label application rate

Product	Plant height			atLEAF			Shoot biomass	Root biomass	Total biomass
	20 days	40 days	60 days	20 days	40 days	60 days			
	Pr>f								
Microgeo 1x vs. 2x	0.659	1.000	0.631	0.833	0.420	0.917	0.194	0.070	0.376
SoilSoup 1x vs. 2x	0.490	0.120	0.482	0.767	0.852	0.129	0.869	0.281	0.717
MicroGro Bioinoculant 1x vs. 2x	0.941	0.249	0.629	0.702	0.336	0.531	0.086	0.582	0.126
MicroLife Humic 1x vs. 2x	0.305	0.678	0.196	0.026 *	0.559	0.875	0.298	0.556	0.382
Nanobind 1x vs. 2x	0.127	0.868	0.084	0.001 *	0.629	0.404	0.356	0.648	0.337
MicroLife 6-2-4 1x vs. 2x	0.215	0.013 *	0.568	0.809	0.787	0.018 *	0.079	0.103	0.049 *

Note. * denotes significant differences, $\alpha < 0.05$.

3.5 Substrate-Induced Respiration (SIR)

Substrate-induced respiration uses the physiological respiration reactions of the microorganisms from the soil to measure microbial activity (Anderson & Domsch, 1978). According to Swaina, Bastiraya, Jitendraa, and Haibrub (2014), the SIR method offers a reliable and easy assessment of the microbial biomass and other aspects of microbial growth in the soil. The use of this method to evaluate the treatments tested in this study showed statistically significant differences (Table 9). Though SIR was positive for 11 of 15 IPNS treatments, only two treatments were significantly higher than the control (Table 10) and responses in general did not follow the same trend as the plant parameters. The two rates of Microgro Supreme Bioinoculant were the only treatments with SIR values greater than the control. Khan et al. (2015) reported a study testing different bioinoculants and vermicompost in combination and alone, where all treatments had higher soil respiration values than the control which did not receive bioinoculants and vermicompost. Moreover, the same study presented soil respiration increment varying from 29.4% to 53.6% over the control value, depending on the treatment.

Application of HA did not significantly affect the SIR results. Hartz and Bottoms (2010) tested the effects of HA on microbial respiration in two different soils containing high and low organic matter. In their study, the addition of HA enhanced microbial respiration only in the low organic matter soil. Therefore, the high organic matter content present in our soil media may have decreased any potential HA influence on microbial respiration.

Table 9. Analysis of variance of the IPNS treatments on substrate-induced respiration

Source	ĈIR
	Pr > f
Rep	0.1012
Treatment	0.00
CV	50.0
SED	2.9

Table 10. Substrate-induced respiration (SIR) values represented by the actual control values and the difference between treatment and control values

Category	Treatment comparison	Difference between treatments and control values
		Substrate-induced respiration (SIR)
		ug CO_2/g dry soil/hour
Biofertilizer	M-C (1x)	-0.50
	M-C (2x)	0.40
	S-C (1x)	0.10
	S-C (2x)	0.20
	MB-C (1x)	9.10
	MB-C (2x)	5.80
Humic	H-C (1x)	-0.70
	H-C (2x)	-0.20
Humic + Biofertilizer	N-C (1x)	0.20
	N-C (2x)	-0.20
	ML-C (1x)	1.00
	ML-C (2x)	1.60
	M+H-C (1x,1x)	0.10
	S+H-C (1x,1x)	0.90
	MB+H-C (1x,1x)	2.90
Actual Control Values (C)		4.50

Note. * denotes significant differences, $\alpha < 0.01$.

4. Conclusions

The individual and combined use of HA and biofertilizers generally increase corn growth and development parameters under the conditions of this study. Though all pots received adequate synthetic fertilizer, the control plants were generally smaller and less vigorous compared to the plants receiving either HA or biofertilizer treatments, but no additive benefit was observed for the integrated practice compared to individual applications. At 40 and 60 days PE the biofertilizer products consistently produced plants that were taller than the control. In general, shoot dry matter was increased by the biofertilizer products, while root dry matter was most positively affected by HA products. Impacts on total biomass were mixed based on contributions of increased root biomass, shoot biomass or both with 11 of 15 treatments exhibiting greater total biomass than the control. Differences in atLeaf chlorophyll meter readings were uncommon for any treatment in our study. However, all treatments had higher fluorometer readings at 20 days PE and higher readings for 13 of 15 treatments at 40 days PE. Although the current study cannot affirm that the conjunctive use of HA and biofertilizers is a better practice than the application of each compound alone, we did find positive benefits from the application of these compounds to corn. Further studies addressing different types and levels of stress and greater stress duration should be conducted to validate these findings and contribute further understanding of the value of the IPNS approach.

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