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The Use of Two Locally Sourced Bio-Inocula to Improve Nitrogen and Phosphorus Cycling in Soils and Increase Macro and Micronutrient Nutrient Concentration in Edamame (*Glycine max*. L.) and Pumpkin (*Cucurbita maxima*)

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Abstract: Soil macro- and micronutrient nutrient availability and their uptake by plants are critically reliant upon an active presence of the soil microbiome. This study investigated the effect of two locally sourced bio-inocula, local effective microorganisms (LEMs) and false-local effective microorganisms (F-LEMs), on plant available nitrogen (N) and phosphorus (P), and the uptake of calcium (Ca), magnesium (Mg), potassium (K), and zinc (Zn) content in edamame (*Glycine max.* L.) and pumpkin (*Cucurbita maxima*) grown in a randomized complete block design with four reps, summer 2017 and 2018, respectively. LEM plots showed greater plant-available N during the first week (edamame season) and fourth week (pumpkin season) after treatment applications. During the pumpkin season, post-treatment plant-available P was greater in both summers in LEM plots. Edamame bean had 19%, 3%, 5%, and 16% greater Ca, Mg, K, and Zn content in LEM plots compared to the Control, respectively. The concentration of K in pumpkin pulp at harvest was 31% higher in LEMs than in F-LEMs, while Mg concentration was 42% higher. Pumpkin pulp and seeds also had 27% and 34% greater Ca and Zn concentrations compared to the Control. Our study suggests that LEMs were effective in solubilizing macro- and micronutrients, which led to increased plant uptake.

Keywords: local effective microorganisms; micronutrient; phosphorus solubilizers; plant-available nitrogen; plant-available phosphorus

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1. Introduction

Primary plant production in most agroecosystems is regulated by nitrogen (N) and the net N mineralized largely depends on the nature of organic amendments used in agricultural management practices [1]. N is one of the major plant growth-limiting nutrients in production agriculture [2]. Next to N, phosphorus (P) is the most essential macronutrient [3] for plant growth and development. Worldwide, soil P is highly deficient across many agroecosystems [4], and crop production is contingent upon the availability of P in soil [5]. One reason is that P is readily complexed and occluded by iron (Fe) and aluminum (Al) at lower pH and calcium (Ca) at alkaline conditions and can become less accessible for plant uptake [6]. In addition, plant nutrient concentration may be declining in several food crops [7] and may result in reduced human nutrition if abundant food supplies are not available [8]. Global nutrient deficiencies of great concern for adequate human nutrition are zinc (Zn), Fe, iodine (I) [9], and of additional concern are Ca and selenium (Se) [10]. Nutrient deficiencies affect close to 2 billion people worldwide, most of them in developing countries [11], while macronutrients (N, P, and K) in soil typically affect the yield and quality of crops such as rice (*Oryza sativa*) [12] and wheat (*Triticum aestivum* L.) [13], and micronutrients such as Zn can also reduce both the yield and quality of crops [14]. Therefore,

shortfalls in crop nutrient concentration indicate a need to formulate strategies to conserve and make macro- and micronutrients more bioavailable for plant uptake [15–17].

The cycling and plant availability of N, P, and K, and micronutrients such as Zn relies upon an abundant, diverse, and active suite of soil microbial communities [18]. In many sustainable agricultural practices, the efficient use of soil amendments such as biochar [19], supply of N, P, (potassium) K, and sulfur (S) from animal manures or organic wastes, serves as an important nutrient source for the microbial population [20,21], plant production, and quality [22]. With the increased interest in soil health, in recent years, there has been a drive to understand and implement various nutrient management strategies involving beneficial microorganisms in soil [23]. A better understanding of the intricacies of soil microbial functional groups to provide nutrients in various agroecosystems may help us to conserve global resources and reduce the incidence of environmental degradation [24,25]. Various rhizosphere-dwelling microorganisms known as plant growth-promoting microorganisms [26] (PGPM) promote plant growth and health by fixing N, solubilizing P, mineralizing macro/microelements (Ca; magnesium, Mg; K; and Zn), excreting phytohormones (directly influence plant growth and metabolism), and resisting pathogens [27–32]. Due to their mutualistic interactions within the root rhizosphere and their positive effects on fortifying the soil microbiome, farmers, industry, and researchers have given considerable attention to the formulation and application of exogenous microorganisms.

Many farmers are using commercial bio-additives, for instance, Effective Microorganisms® (EM^{TM}), Euro Mest-mix® (Mx), and Agrimest® (Am) [33]. EM^{TM} advertises that it has specific strains of beneficial and synergistic microorganisms such as ammonia oxidizers, actinomycetes, lactic acid bacteria, yeasts, and phototrophic bacteria [34]. Throughout Central and South America, a locally sourced bio-inoculate called microorganisms of the mountains (MM), a localized version of EM^{TM}, is made and utilized to suppress pests and odors associated with manures and composts. Research on the efficacy of EM^{TM} has had contrasting results. While significant yield increases in wheat grains amended with EM^{TM} compost were found in one study [35], another found no beneficial effects in crop production [36]. These contrasting results could be due to compatible and incompatible climatic conditions of the study regions, soil properties, and interactions between different microbial communities in EM^{TM} and/or with the native microbial populations where EM^{TM} was applied [36,37]. Thus, with widespread use and few published results, more information is needed to understand the edaphic and agronomic impact of locally produced microbial inoculum.

In our study, we adapted the MM formula for temperate climates which resulted in two bio-inoculates that can be grown and utilized by individual producers. We have designated them as (1) Local Effective Microorganisms (LEMs) and (2) False-LEMs (F-LEMs). The LEM bio-inoculum is gathered from actively decomposing upland forest litter combined with carbohydrate-rich growing media, and it is anaerobically fermented. LEMs contain a wide range of functional microbial groups typical of EM^{TM}, except LEMs are collected from the local environment and can be cultured on the farm; therefore, they easily adapt to the immediate environment [38]. F-LEMs are the same growing media (with baker's yeast, molasses, water, charcoal, and raw milk) used in LEMs, fermented as LEMs were, but without forest floor bio-inoculum. Therefore, we hypothesize that the use of LEM inoculum and LEM-inoculated compost will lead to increased N and P in soil for plant uptake. Additionally, we hypothesize that the use of F-LEM- and LEM-inoculated compost will also enhance the uptake of Ca, Mg, K, and Zn in edamame (*Glycine max*. L.) bean and pumpkin (*Cucurbita maxima* Duchesne) pulp and seed.

Thus, the objective of this study was to investigate the effects of bio-inocula, LEMs or F-LEMs on (a) the plant-available nitrogen and nitrate content of soil (PAN); (b) total phosphorus (TKP), organic phosphorus (OP), and plant-available phosphorus (M1 P) fractions in soil; and (c) on the uptake of P, Ca, Mg, K, and Zn concentrations in edamame (*Glycine max*. L.) bean and pumpkin (*Cucurbita maxima* Duchesne) pulp and seed.

2. Materials and Methods

2.1. Site Description

This study was conducted in the southeastern United States at the J. Phil Campbell Research and Education Center in Watkinsville, Georgia, (33°52′ N, 83°27′ W). The soil at the site is a fine kaolinitic, thermic Typic Kanhapludults in the Cecil sandy loam series with a 2–6% slope (Soil Survey Staff. 2006.). The region has 123 cm average annual rainfall and average minimum and maximum temperatures of 10.4 °C and 22.5 °C, respectively.

2.2. Bio-Inocula: LEM or F-LEM

The bio-inoculum, LEM, was made by collecting the O horizon (actively decaying forest floor material) from healthy, well-established mixed pine and hardwood forests. The LEM inoculum (30 kg bag by volume of O horizon material) was then combined with 23 kg of organic semolina, 250 g of baker's yeast, 10 kg of crushed natural hardwood charcoal, and 4 L each of molasses, antibiotic-free raw goat milk, and chlorine-free water to make a solid mixture. This solid mixture was then placed into a food-grade plastic container and sealed for 6 weeks to allow for anaerobic fermentation. After this first solid-phase fermentation, a liquid-phase fermentation was carried out for at least 2 weeks. For that purpose, 3 kg of solid phase materials were placed into a porous sack and suspended in a sealed 120 L tank with 60 L of water and 4 L of molasses. F-LEM was prepared without the forest microbial inoculum but with the baker's yeast growing media at the same ratio as the LEM.

2.3. Composting Process

Antibiotic-free, organic broiler litter (*Gallus gallus domesticus*) was obtained from a local chicken house for composting (used in the summer of 2018). Liquid bio-inocula (LEM and F-LEM) and the Control were applied on composting broiler litter to maintain a water content of the compost of approximately 0.44 g water/g compost on a dry mass basis. Every 28 days, the bio-inocula (F-LEM and LEM) and Control (equal volume of just water) were applied, and the compost piles were turned during each application to ensure proper aeration and moisture regime. The temperature of the compost piles was monitored regularly.

2.4. Experimental Design and Treatments

Experimental plots (6 m × 6 m) were arranged in a randomized complete block design (RCBD) with four replications of each of the following three treatments: (1) LEM, (2) F-LEM, and (3) Control. When LEM and F-LEM were applied in liquid form, an equal amount of untreated water was applied to the Control plots. In summer 2017, we grew edamame (*Glycine max* L.) for 75 days and in 2018 we grew pumpkin (*Cucurbita maxima* Duchesne), and the pumpkins were grown for 61–86 days. In summer 2017, only bio-inocula treatments were surface-applied directly below edamame plants, approximately three weeks after crop emergence at a rate of 2 L liquid m^{-2}. In summer 2018, composted broiler litter inoculated with each of the three treatments was applied to provide 50 kg P ha^{-1} and tilled. In addition to the inoculated compost application, bio-inocula treatments were surface-applied (2 L liquid m^{-2}) directly below pumpkin plants after emergence, at the contact point of the stem and the soil. The nutrient application rates are detailed in Table 1.

In summer 2017, after tilling with a BCS rototiller to a depth of 15 cm (BCS America, 14151 Fir Street, Oregon City, OR, USA) and smoothing, edamame was planted (8 July 2017) with a Jang planter (Mechanical Transplanter Company, Holland, MI, USA) at a depth of 2.5 cm with 38 cm spacing. In the summer of 2018, after tilling and smoothing, two mounds (6 m × 0.5 m × 0.1 m) in each plot were made with the help of Tillie (Carts and Tools, Corvallis, OR, USA), and 18 seeds were planted in each mound and thinned to nine plants (0.6 m between plants). The two mounds were separated by 1.6 m and had 1.6 m on either side of the mounds within the 6 × 6 m plots described in Section 2.4. Table 2 describes the workflow for the study.

Table 1. N, P, K, Ca, Mg, and Zn applied from treatments in edamame, summer 2017 (only liquid inoculant applied) and pumpkin, summer 2018 (bio-inoculated compost applied, and liquid inoculant applied), on a dry weight basis.

	Summer 2017 (kg ha^{-1} from Liquid Bio-Inocula)		**Summer 2018 (kg ha^{-1} from Compost)**					
	N	P	N	P	Ca	Mg	K	Zn
Control	ND	ND	80	50	62	24	112	2
F-LEM	ND	ND	74	50	72	28	132	2
LEM	ND	ND	76	50	68	28	126	2

Note: LEM, local effective microorganism, is locally sourced bio-inoculate for the temperate region and is a fermented mixture of actively decomposing leaf litter and a carbohydrate-rich solution; F-LEM is a fermented mixture of only the carbohydrate-rich solution. ND = Not Determined.

Table 2. Workflow describing specific activities undertaken during the study.

Edamame—Summer 2017		**Pumpkin—Summer 2018**	
Month	**Activities**		**Month**
25 June 2017	Baseline soil sampling	Baseline soil sampling	30 June 2018
28 June 2017	Plot preparation	Plot preparation and compost application	5 July 2018
8 July 2017	Edamame planting	Pumpkin planting	5 July 2018
3 August 2017	Liquid bio-inocula application	Liquid bio-inocula application	18 July 2018
11 August 2017 and 5 September 2017	Post-application soil sampling ("1st and 4th"week after application)	Post-application soil sampling ("1st and 4th" week after application)	26 July 2018 and 25 August 2018
21 September 2017	Plant harvested	Plant harvested	4, 11, 20, and 29 September 2018

2.5. Soil Sampling

Baseline soil sampling was carried out prior to treatment application in both summers, as well as after the first and fourth weeks of treatment application each year for several soil chemical and microbial parameters (discussed in detail in Sections 2.6 and 2.8). Soils were sampled at a depth of 0–15 cm using 2.5-cm diameter soil augers (8 to 10 soil cores). The samples were kept refrigerated at 4 °C until they were air-dried, ground, and passed through a 2-mm sieve for further chemical analysis. For microbial analysis, soil samples were immediately separated and kept at −20 °C until the soil genomic DNA was extracted.

2.6. Microbial Community Analysis

Microbial DNA was extracted from liquid LEM and F-LEM inoculum in summer 2017 and from inoculated composts applied in 2018. The resources did not allow us to perform a microbial 16s analysis of 2018 bio-inocula or summer 2018 soil. Samples were extracted using QIAGEN DNeasy PowerSoil Kit (DNeasy PowerSoil Kit Handbook, May 2017, Qiagen, Valencia, CA, USA). Extracted DNA was further amplified using the Ion 16S Metagenomics™ kit to amplify the V4 and V5 hyper-variable regions of bacterial 16S rRNA. Bacterial universal primers 515F (5/-GTGYCAGCMGCCGCGGTAA-3/) and 806R (5/-GGACTACNVGGGTWTCTAAT-3/) were combined with barcoded adapter sequences. The Fusion Method, as described in Ion Amplicon Library Preparation (Ion Torrent Systems Incorporated, Guilford, CT, USA), was used to prepare the sample library. The Ion PGM™ Hi-Q™ sequencing kit was used to load the enriched samples on a 316™-chip and sequence them in the Ion PGM™ sequencer (Ion Torrent Systems Incorporated, Guil-

ford, CT, USA). Sequence reads were analyzed by Ion Reporter™ against two reference databases, MicroSEQTM(R) 16S Reference Library (v2013.1) and Greengenes (v13.5). The sequencing was performed in Coastal Palins Soil, Water, and Plant Research Center, United State Department of Agriculture Agricultural Research Service, Florence, South Carolina.

2.7. Soil Respiration (CO_2) and Ammonia (NH_3) Volatilization

CO_2 respiration and NH_3 volatilization were measured from each plot in Summer 2017 and 2018. Measurements were made on days 0 (the day before application), 1 (immediately following application), 3, 7, 14, and 28 after each treatment application. In each plot, two static gas trap chambers (20-cm ID PVC pipe open at the bottom containing two metal rings inside with airtight removable lid) were utilized on each sampling date. Within each of the chambers, the two metal rings held glass jars, one with alkali (100 mL 2 M NaOH) to trap CO_2 and one with acid (100 mL 1 M H_3BO_3) to trap NH_3. The chambers were inserted 5 cm into soil, and alkali and acid traps were placed into the metal rings and the lids were closed for 24 h. After 24 h, the jars were collected and brought back to the lab for chemical analysis. CO_2 was measured by placing a 10 mL aliquot from the glass jars into centrifuge tubes and adding 8 mL $BaCl_2$ followed by centrifugation (1500 rpm for 3 min) and subsequent back-titrating of the aliquot with 1 M HCl [39]. NH_3 was measured by the change in pH of the H_3BO_3 in the glass jars followed by back titration with 0.0025 M HCl to the initial pH of 1 M H_3BO_3 (4.8 to 5.0) [40].

2.8. Soil Analysis

Soil pH was determined in water in a ratio of 1:5 (soil/water) for all soil-sampling phases. Baseline soil samples along with 1st week and 4th week soil samples after treatment applications were extracted for plant-available nitrogen (PAN, the sum of ammonium (NH_4^+) and nitrate (NO_3^-)) by using the cold KCl (2 M) extraction method [41]. All extractants were frozen until they could be analyzed spectrophotometrically on a Tecan Infinite® 200 PRO Series (Tecan Trading AG Switzerland). The NH_4^+ content was determined by using the salicylic acid method [42] and the NO_3^- content was determined by the vanadium (III) chloride method [43]. Plant-available nitrogen was obtained by adding NH_4^+ and NO_3^- fractions. Phosphorus fractions, total Kjeldahl P (TKP), organic P (OP), and Mehlich-1 soil P (M1 P) were analyzed on Baseline and 4th week soil samples. TKP was determined using the Kjeldahl method [44], M1 P was measured in the Mehlich-1 extract [45], and OP was determined by ashing soil samples at 550 °C for 1 h followed by H_2SO_4 (0.5 M) extraction [46]. The P from each of the extractions was determined by a modified single-solution blue dye method [47] using Tecan Infinite® 200 PRO Series, 2014 (Tecan Group Ltd., Männedorf, Switzerland).

2.9. Edamame and Pumpkin Productivity

Edamame was hand-harvested from the inside eight rows and the fresh bean and pod weight were recorded. Above-ground biomass for each plot (inside eight rows) was also harvested and weighed and dried at 60 °C for 48 h. The stabilized dry weights were recorded and used in the dry matter (DM) correction of fresh weights [48]. Pumpkins were harvested for a total of 4 consecutive weeks. Individual pumpkins were carefully hand-harvested based on their maturity and ripening. Pumpkin weight, circumference, and stem length were recorded each week immediately after each harvest. From each harvest, two pumpkins were randomly selected for each treatment for elemental analysis.

2.10. Nutritional Value of Edamame and Pumpkin

Edamame beans from summer 2017 were freeze-dried and then ground using a mortar and pestle. Pumpkins were cut into eight individual pieces, seeds removed, and stored at −20 °C until analysis. Two of the eight pieces were digested for the elemental content of pulp. Pulp was ground with a Ninja Professional 1000 W blender (Shark-Ninja Operating LLC, Needham, MA, USA). Prior to elemental analysis, the pulp and seed were oven-

dried at 65 °C for 48 h. Seeds were ground with a mortar and pestle. Ground edamame bean, pumpkin pulp, and seed were digested via the Kjeldahl digestion method [44]. The digestates were analyzed for Ca, K, Mg, and Zn by an atomic absorption spectrometer, model AAnalyst200 (Perkin Elmer, 11695 Johns Creek Parkway, Suite 150, Johns Creek, GA, USA, 30097-188.1) at 423 nm, 766 nm, 285 nm, and 214 nm, respectively. Digestants were determined spectrophotometrically with the Tecan Infinite® 200 PRO Series (Tecan Group Ltd., Männedorf, Switzerland) following a modified single-solution blue dye method [47].

2.11. Statistical Analysis

Each variable listed below was tested for normality with the Normal Quantile plot and further with the Shapiro–Wilk test for normality in Jmp Pro software and was found to be normally distributed. One-way analysis of variance with Jmp Pro 13 software [49] was used to determine differences between soil pH, soil PAN, TKP, M-1P, OP, and the nutrient concentration (Ca, Mg, K, and Zn) of edamame and pumpkin ($p < 0.05$). Comparisons among multiple means of each parameter between treatments in a crop year were performed using Tukey's honestly significant difference ($p < 0.05$).

3. Results and Discussion

3.1. Microbial Communities in Liquid Bio-Inocula and Compost Inoculated with Liquid Bio-Inocula

Although at varying percent abundance, in both years, major P-solubilizing and N-utilizing bacterial families were found in liquid LEM, F-LEM, and LEM- and F-LEM-inoculated compost (Table 3). In 2017, while Liquid F-LEM was dominated by only Lactobacillaceae, which is to be expected because the liquid F-LEM had baker's yeast and raw goat milk in it, liquid LEM had a diverse mix of bacterial families (Table 3). In 2018, we found N-utilizing bacterial families such as Bradyrhizobiaceae, Chromatiaceae, and Rhodospirillaceae in all inoculated composts at varying percent abundance (Table 3). In addition, in 2018 compost, we found phosphorus-solubilizing bacteria (PSB) families such as Pseudomonadaceae and Bacillalaceae in greater abundance in LEM- and F-LEM-inoculated compost compared to Control compost (Table 3). The greater abundance of P-solubilizing bacterial phyla such as Proteobacteria and Firmicutes and families such as Rhodospirillaceae, Pseudomonadaceae, and Bacillalaceae [50,51] in liquid LEM compared to liquid F-LEM, and in LEM- and F-LEM-inoculated compost compared to Control compost (Table 3), indicates an increased redundancy in microbial functions [52,53]. Functional redundancy is important because if due to biotic and abiotic stress in the soil environment, certain microbial species are dormant or lost, and so there will be ample other microbial species that can carry on ecosystem functioning [54,55].

3.2. Soil pH, NH_3 Volatilization, and CO_2 Respiration from the Soil in Edamame, Summer 2017, and Pumpkin, Summer 2018

In both growing seasons, at the first week, F-LEM and LEM had significantly lower soil pH compared to the Control ($p < 0.05$); however, by the 4th week, no significant differences between treatments were observed (Figure 1a,b). The greater presence of PSBs (*Acetobacteraceae*, *Burkholderiaceae*, and *Lactobacillaceae* in edamame season; *Pseudomonadaceae* and *Bacillalaceae* in pumpkin season) applied through liquid LEM and compost inoculated with LEM (Table 3) is reported to produce a wide range of organic acids, such as gluconic acid, succinic acid, maleic acid, and lactic acid in soil [56,57], which may have contributed to the low soil pH measured in the LEM and F-LEM plots. Consequently, this low soil pH may have facilitated higher PAN at the initial stages of the field experiment (discussed in the following section).

Table 3. Phosphorus-solubilizing and nitrogen-utilizing microbial communities in applied liquid LEM and F-LEM inoculum in summer 2017; LEM-, F-LEM-, and Control (non-chlorinated water)-treated composts in summer 2018. Abbreviations for bacterial functions within LEMs and F-LEMs are as follows: AOB, ammonia-oxidizing bacteria; BC, bio-controller; N-fix, nitrogen fixing bacteria; PrslfB, purple sulfur bacteria; PSB, phosphorus-solubilizing bacteria; and Abs, Absent. Percent abundance of order is given in parentheses for respective families.

Phylum	Class	Order	Family	F-LEM (2017)	LEM (2017)	Control Compost (2018)	F-LEM Compost (2018)	LEM Compost (2018)
Proteo-bacteria	α-Proteobacteria	Rhizobiales	*Hyphomicrobiaceae* (Pht)	Abs	41 (1)	16 (33)	100 (0.2)	100 (0.6)
			Bradyrhizobiaceae (N-fix)	Abs	59 (2)	Abs	Abs	Abs
		Rhodospirillales	*Rhodospirillaceae* (N-fix)	Abs	43 (0.5)	100 (5)	100 (2)	100 (1)
			Acetobacteraceae (N-fix) (PSB)	Abs	57 (0.7)	Abs	Abs	Abs
	β-Proteobacteria	Burkholderiales	*Burkholderiaceae* (PSB)	Abs	100 (1)	Abs	Abs	Abs
	γ-Proteobacteria	Pseudomonadales	*Pseudomonadaceae* (PSB) (BC)	Abs	Abs	75 (4)	100 (60)	100 (53)
			Chromatiaceae (PrSlfB) (AOB)	Abs	Abs	28 (2)	65 (0.2)	72 (0.4)
Firmicutes	Bacilli	Lactobacillales	*Lactobacillaceae* (PSB)	100 (99)	99 (97)	Abs	Abs	Abs
		Bacillales	*Bacillalaceae* (PSB) (BC)	Abs	95 (95)	47 (26)	60 (21)	60 (20)

Note: LEM, local effective microorganism, is a locally sourced bio-inoculate for the temperate region and is a fermented mixture of actively decomposing leaf litter and a carbohydrate-rich solution; F-LEM is a fermented mixture of only the carbohydrate-rich solution.

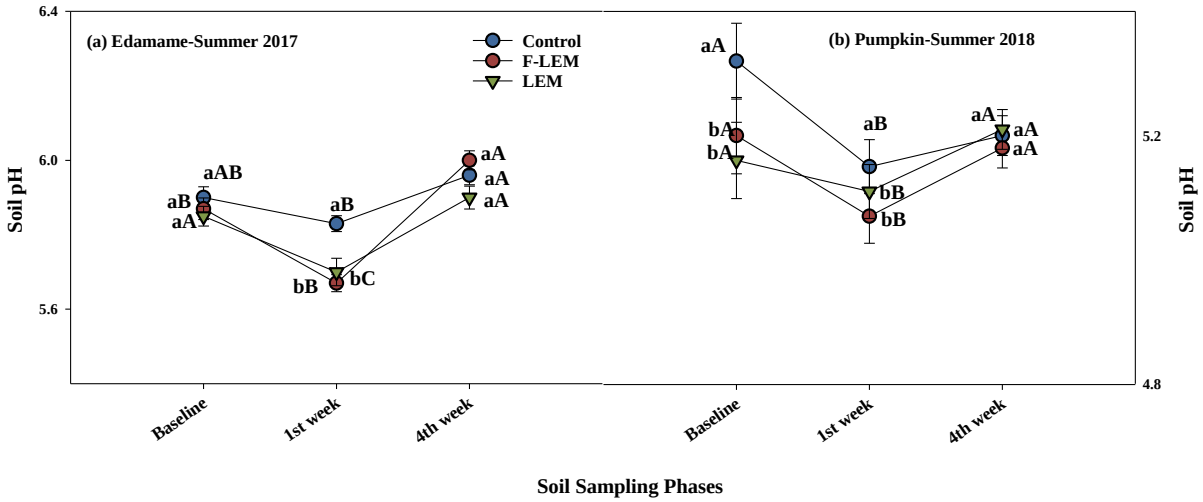

Figure 1. Soil pH (0–15 cm) in different soil sampling phases in (**a**) edamame, summer 2017, and (**b**) pumpkin, summer 2018. Different lower-case letters indicate significant difference ($p < 0.05$) between treatments on a sampling date and different capital letters indicate significant difference ($p < 0.05$) between soil sampling dates for a given treatment.

In both growing seasons, cumulative soil respiration (kg CO_2 ha^{-1}) was not statistically significant between treatments. The cumulative loss of NH_3 was significantly greater from the Control plots compared to F-LEM and LEM ($p < 0.10$; Figure 2a and $p < 0.05$; Figure 2b)

on Day 28. The overall low cumulative loss of NH_3 (ranging from 1 to 5 mg N m^{-2}) is likely attributed in a large part to the overall low soil pH [58] because significant NH_3 usually occurs above pH 7. The greater abundance of *Rhodospirillaceae* and *Chromatiaceae*, in addition to the soil pH, in LEM liquid and compost inoculated with compost may have influenced the lower NH_3 volatilization in LEM plots [59,60].

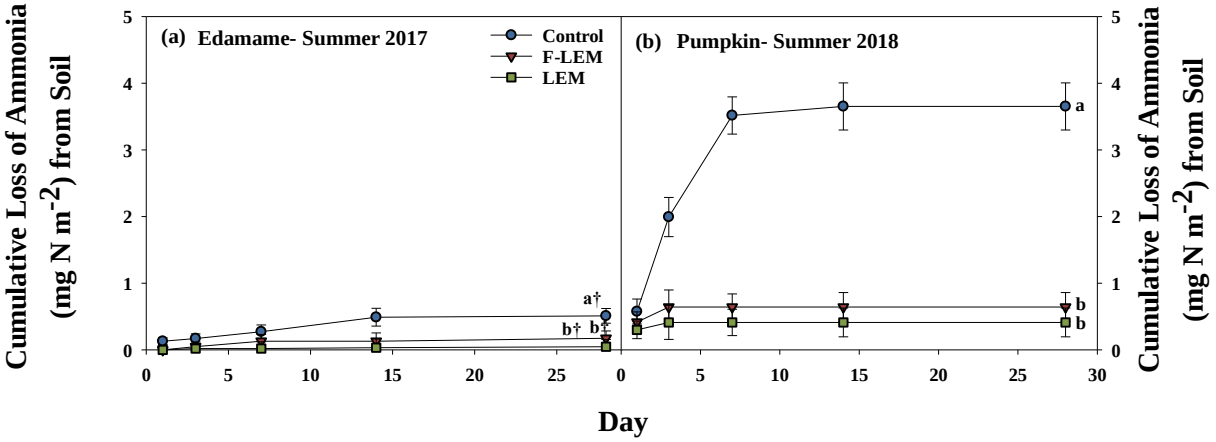

Figure 2. Cumulative ammonia loss (mg N m^{-2}) from the soil in (**a**) edamame, summer 2017, and (**b**) pumpkin, summer 2018. Different lower-case letters indicate significant differences at $p < 0.05$ between treatments on a sampling date and different lower-case letters with † indicate significant differences at $p < 0.10$ between treatments on a sampling date.

3.3. Soil-N Fractions

The microbially driven mineralization of organic forms of N to NH_4^+ is of major significance to N availability in soil for plant uptake [61,62]. In the edamame, summer 2017, the 1st week's soil samples from LEM plots had significantly greater amounts of N mineralized (greater PAN in LEM) compared to both Control and F-LEM plots ($p < 0.05$) (Figure 3a). In the 4th week, PAN decreased significantly from the 1st week, however, with no significant differences between treatments. In the 4th week, although NO_3^- was also significantly lower than at Baseline and during the 1st week, the differences between treatments were not significant (Figure 3b). In the pumpkin, summer 2018, soil PAN also decreased significantly from Baseline to the 1st and 4th weeks (Figure 3c). In the 4th week, PAN was significantly greater in LEM plots than in either the Control or F-LEM plots (Figure 3c). A similar trend in both PAN and NO_3^- content in the Control was observed possibly due to the addition of moisture into the soil which potentially acted as a catalyst for optimum microbial activities of the native soil microbiome [63]. In this study, the direct application of bio-inocula in the liquid form has provided microbial consortia (LEM and F-LEM) that can easily move into the soil and may have prompted rapid mineralization of the labile organic matter during the 1st week of application [38,64]. The subsequent inorganic nitrogen assimilation by plants was reflected in the lower PAN in the 4th week.

3.4. Phosphorous Fractions of Soil

PSBs play a crucial role in solubilizing fixed P into plant-available P forms. It has been reported that one of the most important traits of plant growth-promoting microbial consortia is mineral solubilization and consequent nutrient availability for plant uptake [65–68]. Regardless of treatments, in both summers, soil TKP and OP content decreased significantly ($p < 0.05$) at the 4th week from Baseline (Figure 4a,b). In edamame, summer 2017, M1 P in the 4th week was significantly greater in the LEM treatments (75% greater) compared to either Control or F-LEM (Figure 4a). In pumpkin, summer 2018, with M1 P in 4th-week soil, LEM was about 38% greater than the Control (Figure 4b) ($p < 0.05$). The addition of PSBs (predominately *Pseudomonadaceae*, *Bacillaceae*, and *Lactobacillaceae*; Table 3) through both liquid inoculum (applied in the summer of 2017) and inoculated compost in conjunction

with the liquid inoculum (applied in the summer of 2018) played key roles in solubilizing soil P and therefore making the P more plant-available [69–71]. Additionally, the observed significant decrease in TKP in this study is suggestive of P mineralization and solubilization by PSBs (Figure 4a), and may have resulted in two P pools, primarily OP, which, upon incorporation into microbial biomass, can act as a potential P source for the next crop rotation [72], and, secondly, M1 P, a significant source of plant nutrients.

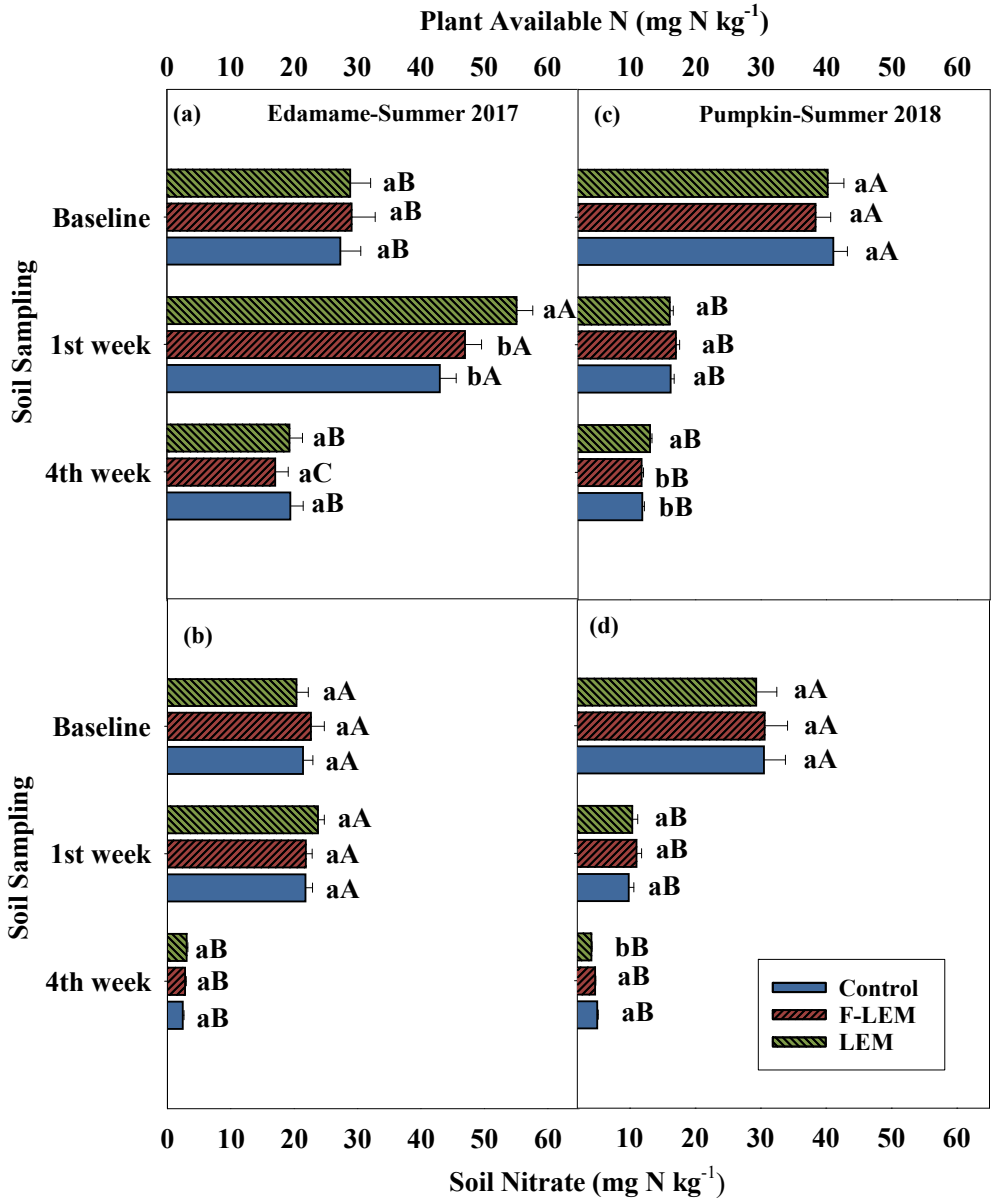

Figure 3. (**a**,**c**) Plant-available nitrogen and (**b**,**d**) nitrate content measured as NO_3-N content in soil (0–15 cm) in edamame, summer 2017, and in pumpkin, summer 2018, respectively. Different lower-case letters indicate significant differences ($p < 0.05$) between treatments on a sampling date and different capital letters indicate significant differences ($p < 0.05$) between soil sampling dates.

3.5. Edamame and Pumpkin Productivity; Nutritional Value of Butterbean Edamame and Pumpkin

This study did not find any significant differences between treatments in edamame biomass and yield or in cumulative pumpkin productivity (Table 4).

However, we found significant differences in both bean and pumpkin nutrient concentrations (Table 5).

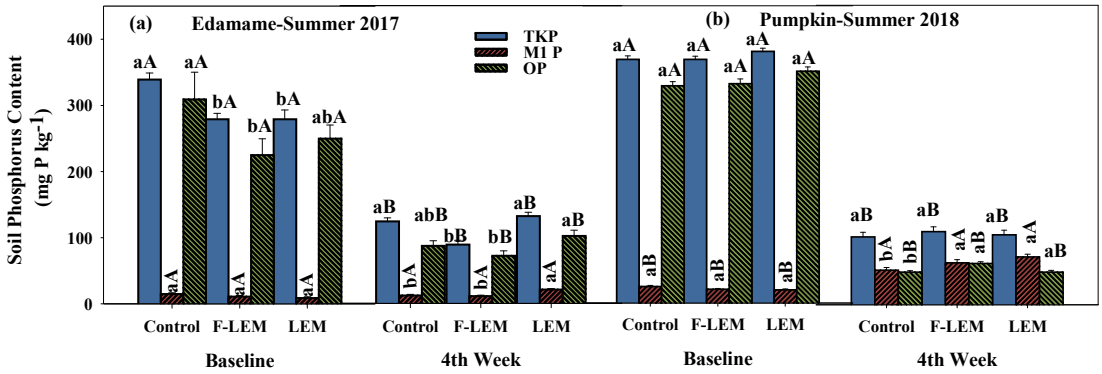

Figure 4. Total Kjeldahl phosphorus (TKP), plant-available phosphorus, measured as Mehlich−1 (M1 P) and organic phosphorus (OP) in soil (0–15 cm) during (**a**) edamame, summer 2017, and (**b**) pumpkin, summer 2018. Different lower-case letters indicate significant differences ($p < 0.05$) between treatments on a sampling date and different capital letters indicate significant differences ($p < 0.05$) between soil sampling dates.

Table 4. Edamame yield (kg ha^{-1}) in edamame, summer 2017, and cumulative yield (yield was recorded for 4 consecutive weeks during growth season) of pumpkin in pumpkin, summer 2018, were recorded on a dry weight basis.

Treatment	Edamame Biomass [kg ha^{-1}]	Edamame Beans [kg ha^{-1}]	Cumulative Pumpkin Yield [kg ha^{-1}]
Control	3395 a [166]	4568 a [1573]	2455 a [274]
F-LEM	3052 a [209]	5381 a [1769]	2035 a [336]
LEM	2838 a [517]	5006 a [1650]	2026 a [243]

Note: Lower letters indicate significant differences between treatments ($p < 0.05$), and mean standard error is given in brackets.

Table 5. Phosphorus, calcium, magnesium, potassium, and zinc concentrations of butterbean edamame and pumpkin pulp and seeds (mg per 100 gm dry weight). Lower rows are percent increase (+) or decrease (−) for LEM relative to Control; C to L, and LEM relative to F-LEM; F to L.

	Edamame Beans [mg/100 g]					Pumpkin Pulp [mg/100 g] (Cumulative of 4 Weeks)					Pumpkin Seeds [mg/100 g] (Cumulative of 4 Weeks)				
	P	Ca	Mg	K	Zn	P	Ca	Mg	K	Zn	P	Ca	Mg	K	Zn
Control	289 a [13]	44 b [3]	156 b [2]	562 b [9]	5 b [0.043]	13 b [1]	140 b [7]	650 ab [38]	692 ab [41]	2 b [1]	273 b [2]	462 b [38]	683 ab [55]	828 ab [595]	15 b [1]
F-LEM	283 a [11]	47 ab [2]	157 b [1]	525 c [5]	5 b [0.070]	9 b [3]	139 b [9]	494 b [81]	563 b [94]	2 b [3]	270 b [21]	543 b [155]	535 b [84]	591 b [822]	18 b [1]
LEM	266 a [17]	54 a [2]	161 a [1]	591 a [5]	6 a [0.067]	25 a [5]	193 a [18]	856 a [60]	820 a [45]	3 a [2]	342 a [21]	700 a [204]	854 a [60]	1077 a [82]	27 a [3]
C to L(%)	−8	+23	+3	+265	+20	+92	+38	+32	+18	+50	+25	+52	+25	+30	+80
F to L(%)	−6	+15	+3	+13	+20	+178	+39	+73	+46	+50	+27	+29	+60	+82	+50

Note: Lower letters indicate significant differences between treatments ($p < 0.05$), and mean standard error is given in brackets.

In edamame, summer 2017, nutrient concentration was consistently higher in LEM-inoculated edamame beans compared to Control edamame beans. The concentrations of Ca (19%), Mg (13%), K (5%), and Zn (16%) were significantly higher in LEM edamame

($p < 0.05$) compared to Control edamame (Table 5). Except for the Ca concentration of the edamame bean, LEM inoculum also performed significantly better compared to F-LEM inoculum (Table 5). The P concentration of edamame bean was not significantly different between treatments. In the summer of 2018, pumpkin pulp had greater cumulative Mg (856 mg/100 g) and K (820 mg/100 g) content when grown with LEM compared to F-LEM (494 and 563 mg/100 g, respectively). Cumulative Mg and K were also significantly higher ($p < 0.05$) in LEM seeds (854 and 1077 mg/100 g, respectively) than F-LEM (535 and 591 mg/100 g, respectively) (Table 4). Furthermore, LEM inoculation also facilitated increased uptake of P, Ca, Mg, K, and Zn in pumpkin pulp and seeds. Ca and Zn concentration in LEM-inoculated pumpkin pulp was 27% and 33% greater ($p < 0.05$) than the Control (Table 5), respectively. Pumpkin seeds also accumulated significantly greater Ca (34%) and Zn (44%) in LEM-inoculated plots ($p < 0.05$) than in the Control (Table 5), respectively. Additionally, significantly greater concentrations of Mg and K in pumpkin pulp and seeds were observed in LEM inoculum compared to F-LEM inoculum (Table 5). Significantly higher P concentration in pumpkin pulp and seed in LEM-inoculated plots can partly be explained by greater M1 P in LEM plots by the 4th week (Figure 4b). When considering just the pumpkin pulp, the effect of LEM increased the P concentration by 92% compared to the Control pumpkin pulp, while the M1 P only increased by 38% (Table 5). Furthermore, PSBs have the potential to release organic acids such as gluconic acid, succinic acid, or indole acetic acid in soil, and subsequently lower the soil pH [73], thereby helping to release complex macro- and micronutrients for plant use. Therefore, in both summers, the greater abundance and complex diversity of PSBs (Table 3) applied through LEM (in 2017, applied only as liquid, and in 2018, applied with inoculated compost and liquid both) have enhanced the micronutrient, for instance, Zn uptake by plants from the soil in LEM plots [50,71]. An interesting fact to note would be the performance of the F-LEM treatment in this study. Predominately Lactobacillus or Bacillus, F-LEM helped to improve Ca density in edamame (like LEM) compared to the Control, possibly by mineral solubilization.

4. Conclusions

The use of LEMs as plant-growth-promoting microbial consortia did increase the plant-available forms of N and P in these Southeastern USA soils when used for growing edamame (*Glycine max*. L.) and pumpkin (*Cucurbita maxima*). LEMs offer functional redundancy through numerous nitrogen-utilizing and phosphorus-solubilizing microbial communities. A repeated and direct application of Local Effective Microorganisms after plant emergence can be recommended for enhanced plant-available nitrogen and phosphorus in soil for both edamame- and pumpkin-growing systems. Zn is a prominent nutrient that faces a shortfall in global human nutrition, and recent reviews have shown that higher atmospheric CO_2 may result in Zn deficiency in beans [74]. In our study, the resulting greater concentration of Zn in plant parts through Local Effective Microorganism application may offer an easy and affordable way of reducing requirements for nitrogen, phosphorus, potassium, and zinc through fertilizer or compost by making more effective use of these nutrients already in the soil. In conclusion, although amending with a diverse bio-inoculum such as LEM did not increase yields, it did increase the nutrient concentrations while maintaining plant productivity in the iron-rich soils of the Southeastern United States.

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