Article

Organic Materials and Their Chemically Extracted Humic and Fulvic Acids as Potential Soil Amendments for Faba Bean Cultivation in Soils with Varying CaCO₃ Contents

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Abstract: Organic amendments are important sources of nutrients that release upon organic matter degradation, yet the stability of these organics in arid and semi-arid regions is relatively low. In contrast, humic substances (HS) are resistant to biodegradation and can keep nutrients in the soil available for the plant over a long time. Combinations between humic substances (HS) and mineral-N fertilizers are assumed to retain higher available nutrients in soils than those recorded for the sole application of either mineral or organic applications. We anticipate, however, that humic substances might not be as efficient as the organics from which they were extracted in increasing NP uptake by plants. To test these assumptions, faba bean was planted in a pot experiment under greenhouse conditions following a complete randomized design while considering three factors: two soils (calcareous and non-calcareous, Factor A), two organics (biogas and compost, Factor B) and combinations of the organics and their extracts (HA or FA) together with complementary doses of mineral-N $((NH4)_2SO_4)$ to attain a total rate of 50 kg N ha^{-1} (the recommended dose for faba bean plants) (Factor C). Results indicated that nitrogenase activity increased significantly due to the application of the used organics. In this respect, compost manure caused higher nitrogenase activity than biogas manure did. Humic substances raised NP-availability and the uptake by plants significantly; however, the values of increase were lower than those that occurred due to the compost or biogas manure. Moreover, the sole application of the used organics recorded the highest increases in plant biomass. Significant correlations were also detected between NP-availability, uptake and plant biomass. This means that HS could probably retain nutrients in available forms for long time periods, yet nutrients released continuously but slowly upon decomposition of organics seemed more important for plant nutrition.

Keywords: humic substances; sandy soils; faba bean; NP-uptake; NP-availability

1. Introduction

Faba bean (*Vicia faba* L.) is an important legume crop in Egypt [1] that belongs to the family Fabaceae [2]. Its seeds are the edible parts that may partially replace meat and dairy products in the human diet [3] because they are rich in complex carbohydrates, proteins,

dietary fiber and several bioactive compounds [4]. This crop can be grown successfully on poor light-textured soils [5]; yet these soils need major improvements to raise their productivity [6,7]. Accordingly, organic applications are guaranteed to attain these aims [8]. These amendments not only improve soil physical and chemical characteristics [9–12] but also chelate nutrients [13] and increase their availability, especially in alkaline and calcareous soils [14]. Even in acid soils, organic amendments can maintain the optimal pH for nutrient availability [15].

Moreover, organic amendments lessen nutrients losses through leaching from top layers [16] of light-textured soils [17] and hence increase their uptake by plants [10]. In the case of P, organic amendments can also diminish P-sorption in soil [18]. Despite that, Guppy et al. [19] mentioned that researchers ignored the release of P during organic matter degradation in their calculations, which is the main reason for such reductions in P-sorption. Therefore, this area still needs further investigation.

Humic substances (HS), which comprise up to 70% of soil organic matter (SOM) [20], retain longer in soils [21] and resist, to a higher extent, against biodegradation [22]. Their functional groups increase nutrient retention in soil [23]; consequently, HS can be used as nutrient reservoirs [24]. This may, in turn, lessen P-sorption in soil [25]. This study explored the impacts of easily decomposed organic matter versus the more resistant organics (humic and fulvic acids that act as chelates). It is thought that organic amendments, which are highly decomposable in soil, stimulate the activities of beneficial microbiota [26] and also enrich soils with nutrients [13]; however, their stability in arid and semi-arid soils are relatively low [11]. On the other hand, humic substances (HS) are relatively more stable in soil and form coatings on sand particles [27]. This mechanism may play a more significant role in retaining available nutrients within the topsoil via surface complexation, e.g., phosphate [25], especially when these organics are combined with mineral fertilizers [28]. The relatively high molecular weights of the humic substances may, on the other hand, lessen nutrient mobility and therefore decrease their potential loss by leaching from soils [29]. Specifically, we assume that combinations between organic amendments, particularly FA/HA and mineral-N fertilizers, retain higher available P in soils than those recorded for the application of mineral-N or its organic forms when applied solely. Furthermore, these amendments may lead to indirect positive effects on N-availability. Accordingly, these extracts probably increase nutrient availability to extend beyond those attained for the organic amendments themselves (*Hypothesis 1*). Even though the implications of amending soils with humic substances are probably lower than the consequences of the organic amendments on increasing NP-uptake by faba beans because organic amendments act as slow release fertilizers that supply plants continuously with nutrients (*Hypothesis 2*). A controlled greenhouse investigation is recommended to test these hypotheses in order to avoid the consequences of heavy rains (if they occur) on nutrient leaching from the topsoil when this investigation takes place under field conditions.

The current study aims at investigating the effectiveness of using organic amendments from different sources (compost and biogas) and their extracts (HA and FA) for improving NP-availability and uptake by faba bean plants grown on poor fertile light-textured soils (calcareous and non-calcareous ones) for 80 days. Implications of these organic amendments and their extracts on plant growth parameters were also a matter of concern. Results obtained herein might improve our knowledge about the roles of organic amendments in improving nutrients availability in soil; hence, increasing their uptake by plants.

2. Materials and Methods

2.1. Materials of Study

2.1.1. Soils of Study

Surface soil samples (0–30 cm) were collected from both sandy (El Dair, Qalubia Governorate) and calcareous soils (Nubaria, Beheira Governorate, Egypt). These samples were then air-dried, grounded to pass through a 2 mm sieve and analyzed for physical and

chemical properties outlined by Klute [30] and Sparks et al. [31]. The obtained results are presented in Table 1.

Table 1. Physical and chemical properties of the investigated soils.

Property	El Dair (Non-Calcareous Soil)	Nubaria (Calcareous Soil)
Particle size distribution %		
Sand	91.80	94.90
Silt	2.20	2.27
Clay	6.00	2.83
Textural class	Sand	Sand
Soil pH *	7.65	8.20
Soil EC **, dS m^{-1}	1.39	5.00
Organic matter content, g kg^{-1}	3.30	7.10
CaCO$_3$ content, g kg^{-1}	15.00	223.00
Available N, g kg^{-1}	12.70	16.80
Available P, g kg^{-1}	1.35	2.52
Available K, g kg^{-1}	15.50	20.70

* pH was determined in 1:2.5 soil: water suspension; ** EC was determined in soil paste extract.

2.1.2. Organic Manures Source

Compost and biogas manure weas obtained from the Training Center for Recycling Agricultural Residues at Moshtohor (TCRAR), Agricultural Research Center (Egypt). Humic substances were extracted from both amendments as outlined by Sanchez-Monedero et al. [32], i.e., 0.5 *N* KOH was added to the organic amendment, then filtrated, and the supernatants were acidified with HCl to reach pH 2.0 and left overnight. The precipitate, known as humic acid (HA), was separated from soluble fulvic acids by centrifugation at 6000 rpm for 15 min.

Humic acid precipitates were then washed several times with 0.05 *N* H$_2$SO$_4$, then purified by electrodialysis, as Chen et al. [33] mentioned. In the case of fulvic acid, its purification was carried out according to the method described by Kononova [34] and Susilawati et al. [35], i.e., the fulvic acid extract was passed through activated charcoal followed by elution of the charcoal. Then, the solution was transferred to membrane filter and eletrodialyzed until the dialysate was free of Cl-. Elemental analysis for C, H, N and S in organic amendments and their extracts were performed by gas chromatography on a Hewlett-Packard 185 Analytical Center, Faculty of Science, Cairo University. Oxygen was calculated by subtracting total amounts of C, H, N and S (expressed as percentages) from 100. Physical and chemical characteristics of the used organic amendments are presented in Table 2.

Table 2. Elemental analysis of the investigated amendments (compost manure and biogas manure) and their extracted humic (HA) and fulvic (FA) acids.

Character	Compost			Biogas		
	Manure	HA	FA	Manure	HA	FA
C%	49.46	50.71	50.40	51.40	49.90	46.00
N%	2.83	3.10	2.72	2.60	2.85	2.75
H%	1.45	1.93	1.84	2.12	2.10	3.89
S%	3.76	3.96	3.14	3.68	4.15	3.46
O$_2$%	42.50	40.30	41.90	40.20	41.00	43.90
P%	0.23	0.17	0.15	0.30	0.15	0.27
K%	8.82	5.83	6.93	7.97	5.21	6.60
C/N ratio	17.50	16.40	18.50	19.80	17.50	16.70
C/H ratio	34.10	26.30	27.40	24.20	23.80	13.30
C/O ratio	1.20	1.30	1.20	1.30	1.20	1.05
O/H ratio	29.30	20.90	22.80	19.00	19.50	11.30
N/H ratio	1.95	1.60	1.47	1.23	1.36	0.71

2.1.3. Faba Bean Seeds

Seeds of faba bean (*Vicia faba*, c.v Giza-2) were obtained from the Field Crops Research Institute, Agricultural Research Center, Giza, Egypt. These seeds were inoculated with *Rhizobium leguminosarium* (ICARDA441) inoculum using Arabic gum (16%) as an adhesive agent; afterward, seeds were then air-dried for an hour before sowing.

2.2. The Greenhouse Study

A pot experiment was conducted under the greenhouse conditions of the Training Center for Recycling Agricultural Residues at Moshtohor (TCRAR), Qalubia Governorate, Egypt. The mean values of temperature, humidity and daylight during the experimental work were 19.1 °C, 61%, 10.7 h in November, 15.4 °C, 61% and 1.2 h in December, and 18.9 °C, 59% and 10.5 h in January, respectively.

This experiment comprised three factors: (1) soil type (calcareous vs. non-calcareous soil), (2) two organic sources (biogas and compost) and (3) different combinations between the organic amendments and the mineral N, i.e., a control treatment with no organic additions (the recommended dose of N was applied in the mineral form, i.e., 21 mg N kg^{-1} equivalent to 50 kg N ha^{-1}) (T_0), 50% of the recommended dose of N in the organic form plus 50% in the mineral form (T_1), 100% of the recommended dose of N in the organic form (T_2), 5 mL HA kg^{-1} plus a complementary application of the mineral N to satisfy the recommended N dose (T_3), 10 mL HA kg^{-1} plus a complementary dose of the mineral N to satisfy the recommended N dose (T_4), 5 mL FA kg^{-1} plus a complementary application of the mineral N to satisfy the recommended N dose (T_5), 10 mL kg^{-1} of FA plus a complementary dose of the mineral N to satisfy the recommended N dose (T_6). Two kilogram soil portions were mixed thoroughly with any of the abovementioned treatments, in addition to the recommended PK doses, i.e., 31.5 mg P kg^{-1} (equivalent to 75 kg P ha^{-1} as calcium superphosphate, 8.5%P) and 20 mg K kg^{-1} (equivalent to 48 kg K ha^{-1} potassium sulfate, 48%K), while considering PK contents in organic amendments; afterward, soil portions were packed in plastic pots (21 cm diameter × 16 cm depth). Supplementary applications of ammonium sulfate (20.5%N) were considered to bring up N content in all treatments to the recommended dose (equivalent to 50 kg N ha^{-1}). Pots were then arranged, under the greenhouse conditions, in a complete randomized design in triplicates.

Faba bean seeds were cultivated in November 2019 at a rate of five seeds per pot and thinned to four plants per pot two weeks after seed planting. Irrigation was carried out every 5–7 days to bring soil moisture to the field capacity. At the flowering growth stage, the rhizosphere of each pot was sampled to determine nitrogenase (N_2-ase) activity, using the acetylene reduction technique according to Dilworth [36], then determined using gas chromatography (Hewlett-Packard 5890, Wilmington, DE, USA). Plants were removed from soils 80 days after planting, placed on plastic sieves, washed with tap water several times to remove dirt, and rewashed with deionized water twice. Plant materials were then oven-dried at 70 °C for 48 h, and their weights were determined. Soil samples were also collected from the rhizosphere of each pot during plant harvest to measure the available NPK contents.

2.3. Plant and Soil Analyses

Dried plant samples were ground, and samples were digested using a mixture of H_2SO_4 and $HClO_4$ (1:1), as described by Peterburgski [37]. Nitrogen was analyzed in plant digest using the micro Kjeldahl method, while P was determined by ammonium molybdate and ascorbic acid reagents, as outlined by Page et al. [38], then measured by spectrophotometer (Jenway 6705 UV/Vis. Spectrophotometer, Staffordshire, UK). Available N was extracted from the soil by K_2SO_4 (1%), then determined using micro Kjekdahl apparatus in the presence of Devarda alloy, and available P was extracted with $NaHCO_3$ (0.5 N, pH 8.5) and determined calorimetrically, as outlined by Sparks et al. [31].

2.4. Statistical Analysis

Data were statistically analyzed by SPSS statistical software 18 following three-way ANOVA ($p < 0.05$) and Duncan's multiple range tests. All graphs were plotted using SigmaPlot10 software.

3. Results and Discussion

3.1. Effect of the Used Organics and Their Extracts on the Activity of Nitrogenase Enzyme

Biological nitrogen fixation (BNF) is a key reaction of the nitrogen cycle [39], in which the nitrogenase enzyme is the dominant catalyst of dinitrogen reduction [40]. This enzyme changes N_2 to NH_3 [41,42]. Our results indicate that the activity of this enzyme increased significantly owing to organic applications following the order of $T_2 > T_1 \approx T_4 > T_6 > T_3 \approx T_5 > T_0$ (Figure 1). Although the organic component HS was more resistant to biodegradation than the amendments that they were extracted from; yet, HS applications probably increased the decomposition rate of soil organic matter to provide substrates for soil biota [22]. This can effectively enhance their activities, especially the N-fixers [43,44]. Concerning the effect of the organic source on the activity of this enzyme, it was found that compost treatments were more effective than biogas manure ones in enhancing the activity of this enzyme. Generally, the activity of nitrogenase was higher in calcareous than in non-calcareous soil.

Interactions among the abovementioned three factors were of further significant effect on the nitrogenase enzyme activity (Figure 2). In this concern, compost application, at a rate of 100% to satisfy N-needs, recorded the highest increases in the activity of nitrogenase enzyme and generally, organics that originated from compost were more efficient than the corresponding ones that originated from biogas manure in stimulating the activity of such an enzyme in both the sandy calcareous and non-calcareous soil.

3.2. Effect of the Used Organics and Their HA and FA Extracts on Nutrient Availability and Uptake by Plants

3.2.1. Availability and Uptake of N

Application of organic amendments and their extracts raised significantly N availability in soil, which consequently increased its uptake by faba bean plants (Figure 2). The highest increases were attained for the application of either compost or biogas to satisfy 100% of the recommended N-dose (T_2), followed by the application of 50% org-N (T_1), then 10 mL HA kg^{-1} (T_4) or 10 mL FA kg^{-1} (T_6), with no significant variations between the last two treatments. It is well known that these organic amendments are rich in N (Table 2) that was released upon organic matter degradation [6] to increase the availability and uptake of this nutrient by plants [45]. In the case of humic substances, they may effectively decrease ammonium loss through volatilization from the soil [46] because they are negatively charged [47]. Moreover, HS is involved in increasing nitrate transcription to plant roots [48]. Despite that, the HS results were lower than the expected ones. Organics that originated from biogas manure exhibited lower N-uptake than those that originated from compost. This is because compost contained a higher N content than biogas manure (Table 2). Furthermore, ammonium fertilizers underwent oxidation in sandy soil [49]. They also contained more positive functional groups, i.e., NH_2- groups (amines), than those found in biogas manure (Table 2), which can chelate nitrate ions and prevent loss by leaching in light-textured soil [50].

The availability and uptake of N also varied significantly between the two soils under investigation (calcareous vs. non-calcareous soils), where N-availability and uptake were higher in the non-calcareous soil vs. the calcareous one. Probably, N-losses through volatilization increased from the topsoil of the calcareous soil [51]. Moreover, HS was extensively adsorbed via the positively charged amine groups under the alkaline conditions of the calcareous soil [52]; accordingly, N-availability decreased considerably in the calcareous soil vs. the non-calcareous one.

Interactions among the three factors under study also considerably affected N-availability and uptake (Figure 2). Organic treatments significantly increased the availability and uptake of N in both the calcareous and non-calcareous soil, especially compost and biogas manures. Decreasing the level of application of these amendments to a half dose lead to the significant concurrent reductions in both the availability and uptake of N by plants. Generally, the highest increases occurred in the non-calcareous soil when compared with the ones recorded in the calcareous soil.
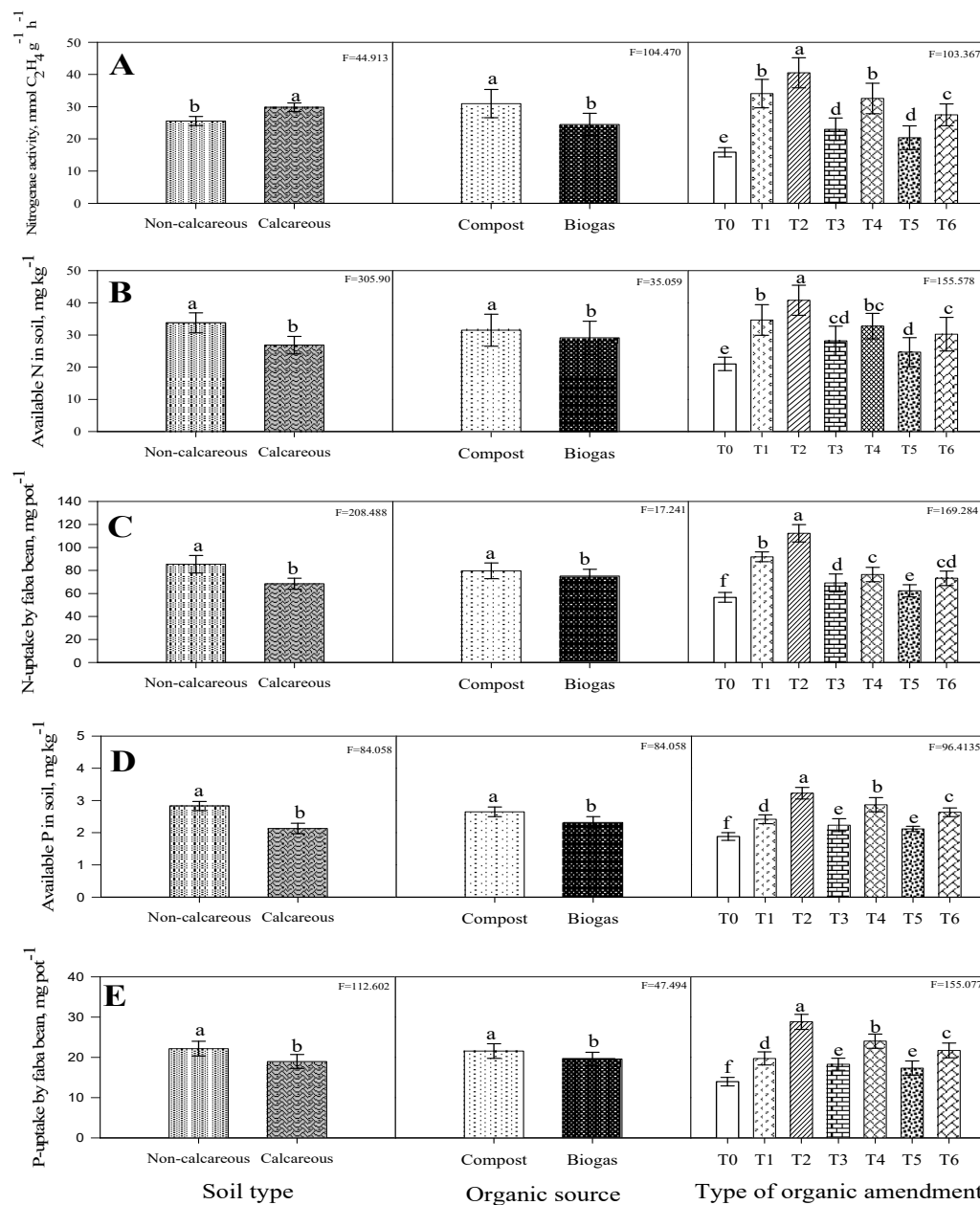

Figure 1. Grand means of nitrogenase enzyme activity in plant rhizosphere (**A**), available N in soil (**B**), N-uptake by faba bean (**C**), available P in soil (**D**) and P-uptake by faba bean (**E**) in light-textured soils as affected by application of organic and mineral N-fertilizers, either solely or in different combinations. Treatments from T_0 to T_6: no organic additions (T_0), 50% org (T_1), 100% org-N (T_2), 5 mL HA kg^{-1} (T_3), 10 mL HA kg^{-1} (T_4), 5 mL FA kg^{-1} (T_5), 10 mL FA kg^{-1} (T_6). Different letters indicate significant variations among treatments.
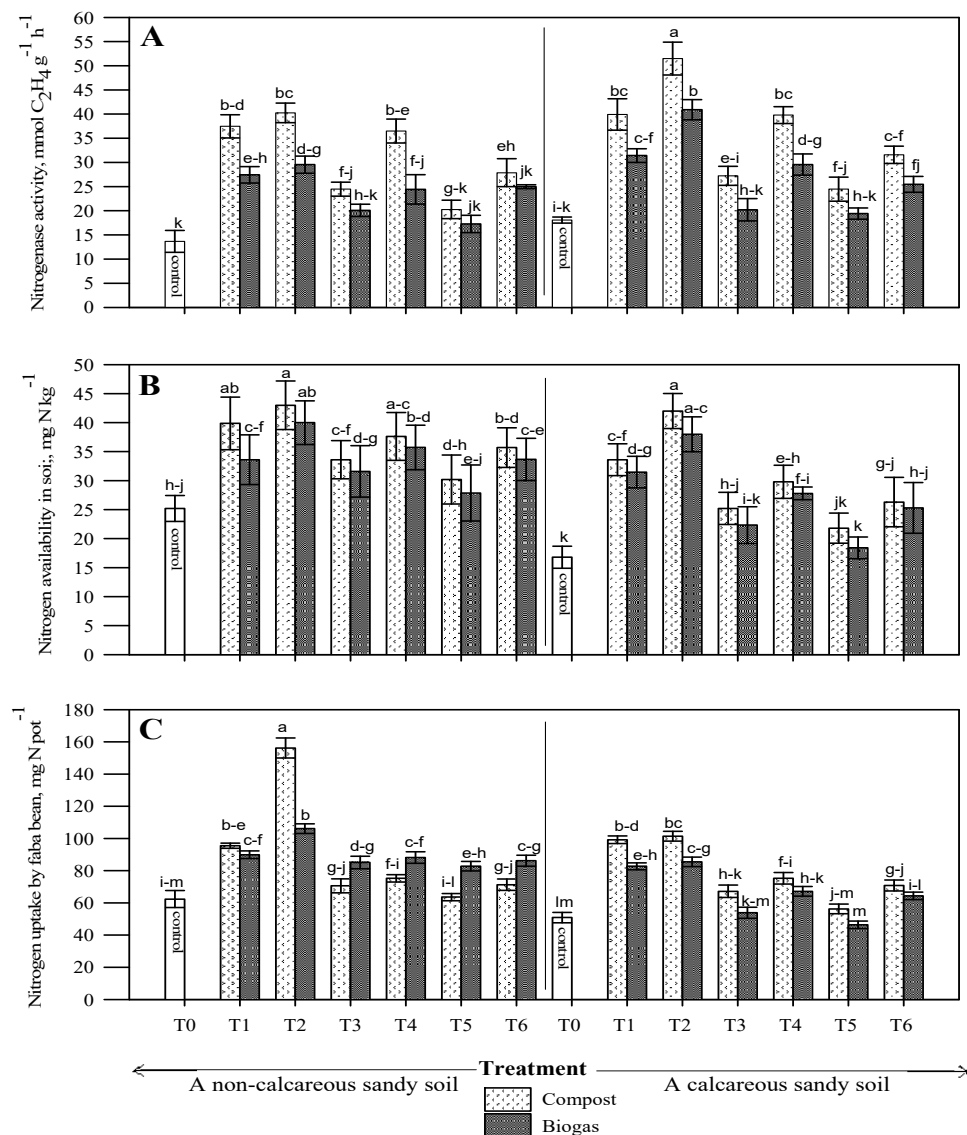
Figure 2. Nitrogenase activity within the rhizosphere (**A**), N-availability in soil (**B**) and uptake (**C**) by faba bean plants as affected by application of organic and mineral N-fertilizers, either solely or in different combinations (mean ± SD). For treatments from T0 to T6, see footnote Figure 1. Different letters indicate significant variations among treatments.

3.2.2. Availability and Uptake of P

The availability of P increased significantly in the two studied soils due to different organic amendments (Figure 2). The highest increases occurred due to the application of either compost or biogas manure to satisfy 100% of the recommended N-dose (T$_2$), with significant superiority for compost vs. biogas manure. Furthermore, the increases in both P-availability and uptake were higher in the non-calcareous soil than in the calcareous one.

The investigated treatments can be arranged according to their effects on increasing available P content in the following descending order: T$_2$ > T$_4$ > T$_6$ > T1 > T$_3$ ≈ (T$_5$) > T$_0$. These findings can be attributed to the ability of organic amendments to form complexes with soluble-P, hence increase P-availability in soil [25] noticeably. In HS, NH$_4^+$ ions found in these extracts might increase their polarity and mobility [53]; hence, adsorb or chelate more ions from the soil. In this concern, it was found that P fractions in light-textured soils existed mainly in association with fulvic and humic acids [54]. Most likely, phospho-humic complexes are formed by bridging with di- and tri-valent cations [55]; however, the effects of HS on P-availability levels were worth the expected ones.

Likewise, P-uptake increased significantly in the non-calcareous soil than in the calcareous one. This uptake varied significantly according to the type of the organic treatment, with a trend similar to that recorded in P-availability. Humic substances are reported to increase apoplast acidification [56] and act as effective redox mediators [57,58] that decrease P-precipitation via changing phosphate species into diprotonated monodentate mononuclear complexes [25]. Though, plants might not be efficient enough to extract P from their organic complexes, probably because P existed in esaphosphate inositol (high-molecular fractions) in soil [55]. Accordingly, it can be deduced that the increases in P-uptake by faba bean plants were mainly attributed to the increases in P released during organic matter degradation rather than the formation of organo-P complexes. These results agree to some extent with Hartz and Bottoms [59], who found that humic acid recorded no significant effect on P-uptake by plants.

Interactions among the three factors under study also significantly affected both P-availability and uptake by plants (Figure 3). It seems that compost was preferred over biogas manure for increasing P-availability when applied as a sole source of N in both the non-calcareous and calcareous soils.
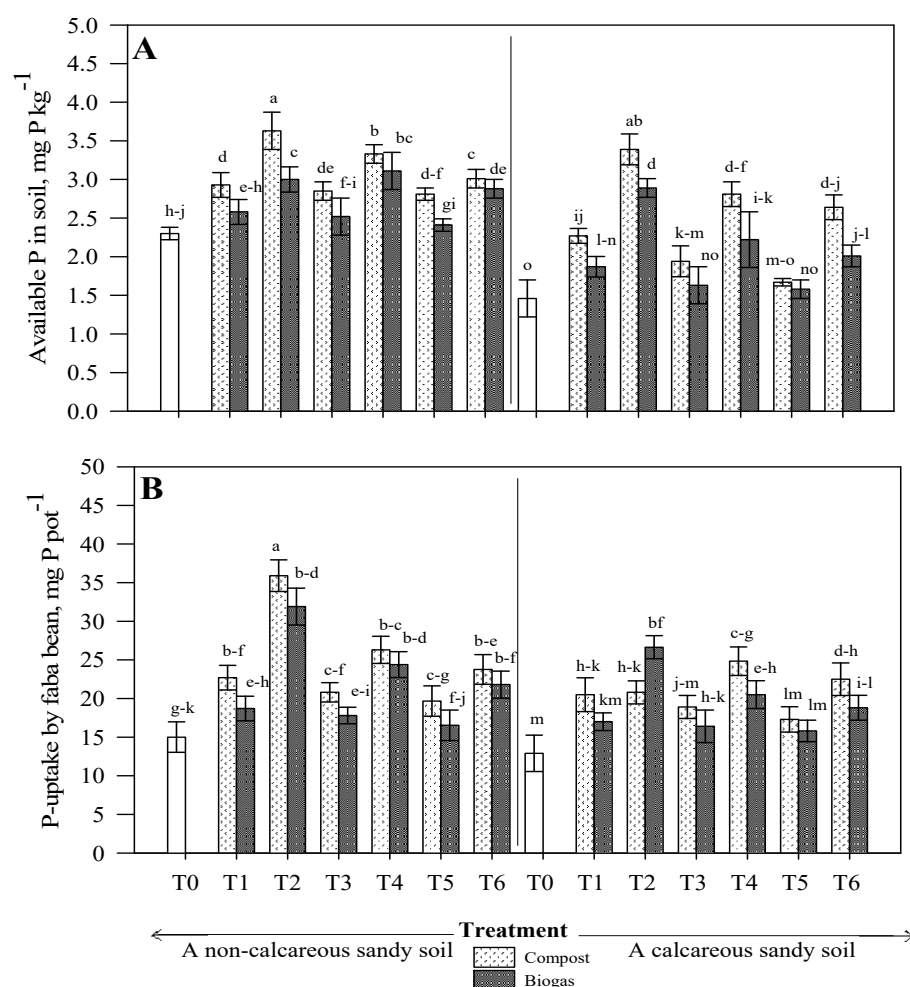

Figure 3. Availability (**A**) and uptake (**B**) of P (mean ± SD) by faba beans as affected by application of organic and mineral N-fertilizer, either solely or in different combinations. Different letters indicate significant variations among treatments.

3.3. Effect of the Used Organics and Their HA and FA Extracts on Plant Growth Parameters

Faba bean dry weights varied significantly owing to the type of applied organic treatment (Figure 4). In this concern, the application of 100% organic amendment (T$_2$) recorded the highest increases in plant dry weights, while the least dry weight value was attained

for the non-amended control treatment (T_0). These results can probably be attributed to the findings that indicate that organic amendments raised soil fertility considerably, especially in both light-textured [10] and calcareous soils [14]. They may also promote plant growth bacteria as well, and this consequently stimulated plant development [60]. The application of humic acid seemed to be preferred over fulvic acid in enhancing plant dry weights, especially with increasing the application rate from 5 to 10 mL kg^{-1}. Although several reports highlighted the implication effects of HS on plant growth [10,61–63], the results obtained herein indicate that these efficiencies were low when compared with the corresponding ones attained due to the application of the organic amendments from which they were extracted (compost and biogas). Most likely, these results highlight the need for the application of higher amounts of HS, which is not a practical agricultural process and at the same time requires additional costs. Furthermore, high application rates of HS may potentially increase the leaching of the soil contaminants to the groundwater and transfer these contaminants to the trophic chain [64].
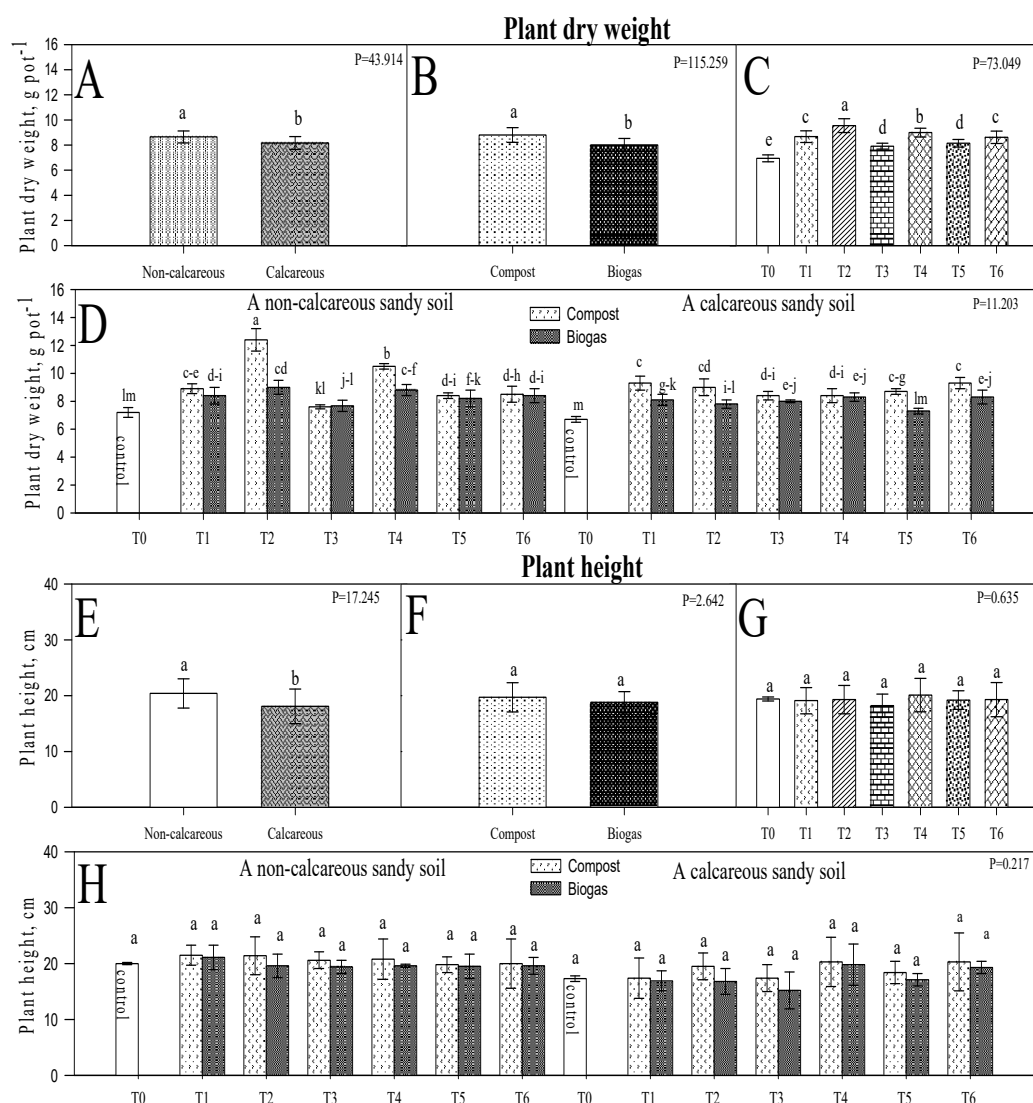

Figure 4. Dry weight (g pot^{-1}) and plant height (cm) of faba beans (mean $\pm$ SD) as affected by application of organic and mineral N- fertilizers, either solely or in different combinations. Effect of different soil types is presented in (**A**,**E**). Effects of different organic sources are presented in (**B**,**F**), while the effects of the different organic treatments are presented in (**C**,**G**). The interactions among the three factors of study are presented in (**D**,**H**). Different letters indicate significant variations among treatments.

Further increases in plant dry weights were attained due to the source of the organic amendment, in which compost treatments recorded higher increases in plant dry weights than biogas manure ones. Generally, plant dry weights were higher in the non-calcareous soil than those in the calcareous one. Interactions among these three factors (soil × organic source × organic treatment) were of further significant effect on plant dry weights (Figure 4), where the highest dry matter yield was recorded for the treatment that received 100% compost in the non-calcareous soil, while the least ones were the non-amended control treatments. On the other hand, plant heights did not vary significantly, whether due to the type of the applied organic source or the organic treatment; however, the effect of the soil type seemed to be significant (Figure 4). Plants grown on the sandy soil were taller than those grown on the calcareous soil. The combination between the three factors was also of no significant effect on plant height.

3.4. A Correlation Study between NP-Availability and Uptake in Relation to Plant Dry Weights and Nitrogenase Activity in Soil

Availability of N, and its uptake, were significantly and positively correlated with each other, and both parameters were highly significantly correlated with the increases that took place in activities of nitrogenase enzyme in soil (Table 3). Likewise, P-uptake by faba bean plants was significantly and positively correlated with P-availability in soil. Furthermore, the increases that occurred in faba bean dry weights were significantly and positively correlated with the concurrent increases that occurred in the uptake of both N and P nutrients. These results highlighted the positive roles of applying light-textured calcareous and non-calcareous soils with organic amendments on stimulating the activities of beneficial N-fixing bacteria, improving NP-availability and uptake by plants and consequently enhancing their growth.

Table 3. Correlation coefficients between NP-availability and uptake in relation to plant dry weights and nitrogenase activity in the soil.

	N-Availability	N-Uptake	P-Availability	P-Uptake	Nitrogenase Activity	Plant Dry Weight
N-availability						
N-uptake	0.834 **					
P-availability	0.796 **	0.777 **				
P-uptake	0.906 **	0.736 **	0.806 **			
Nitrogenase activity	0.715 **	0.633 **	0.654 **	0.597 **		
Plant dry weight	0.582 **	0.649 **	0.667 **	0.544 **	0.545 **	

** Significant at $p < 0.01$.

4. Conclusions

Organic treatments seemed to be more efficient than their extracts (inorganic N-fertilizer) in increasing nitrogen and phosphorus availability in both the calcareous and non-calcareous light-textured soils. Moreover, increasing the application dose of compost or biogas manure (100% rather than 50%) led to significant concurrent increases in nutrient availability. These results did not support *Hypothesis 1*, indicating that combinations between organic amendments, particularly FA/HA and mineral-N fertilizers, increased NP's availability in soils beyond those recorded for the application of either mineral or organic forms when applied solely. Moreover, organic amendments stimulated effectively symbiotic N-fixers and increased the activity of the nitrogenase enzyme. Furthermore, these amendments recorded higher significant increases in NP-uptake and plant growth than their extracts did. These results support *Hypothesis 2*. Most likely, plants and soil biota cannot efficiently utilize nutrients chelated with humic and fulvic acids. Furthermore, it can be deduced that nutrients released continuously but slowly upon the decomposition of organic amendments might be more important for plant growth than those chelated by HS.

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