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The Impact of Legume Biomass as a Soil Amendment for the Production of Barley var. Emerald (*Hordeum vulgare* L.)

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ABSTRACT

Crop production is one of the most important economic activities worldwide and a pillar in achieving food security. One of the different alternatives that have been studied to improve it as well as to reduce its impact on the environment is the substitution of inorganic fertilizers with organic amendments. This study aimed to evaluate the impact of the use of biomass from three different legumes on soil characteristics and the production of barley. To test this, a greenhouse experiment was used to grow barley in soil amended with biomass from alfalfa, soybean, and bean as a single species or a combination of them (biomass of fully grown plants from 30 g seeds). After soil amendment, barley was grown, testing each treatment over 15 weeks through overall growth, amount of grains per spike, and the number of spikes. Moreover, the impact of the amendments on soil characteristics was also evaluated. Results showed that the different amendments significantly modified both soil characteristics and the overall production of barley. Soil organic matter (3.32 %) and organic carbon (1.92%) were significantly higher when soil was amended with a combination of alfalfa and soybean. Further to this, this same treatment consistently produced the largest production and overall plant growth. Based on these results, it can be concluded that the use of plant biomass as an amendment has a significant influence on increasing the total length of barley as well as the number of spikes and seeds per spike while modifying quality.

Keywords: Cereal, soil conditioner, agriculture, alfalfa, soybean

INTRODUCTION

Agriculture is one of the most important economic activities since the origin of humanity. Given this importance, nowadays, there is a general concern regarding the production of enough crops for both human consumption and the manufacturing of a range of goods. As such, an increasing interest in the different alternatives to increase crop production in a sustainable manner has led to the development of several studies with different approaches being constantly tested (Caniato *et al.*, 2020; Boanares *et al.*, 2023). Specifically, the production of cereals such as wheat, and barley represents a key role in different aspects of human well-being as food, livestock fed and even for biofuels (Mittal, 2022). Nonetheless, reports suggest that the production of these crops has been significantly affected by climate change (Mittal, 2022; Farooq *et al.*, 2023). More importantly, in tropical and subtropical countries, the impact that the modification of these climatic patterns has been especially severe (Corlett, 2014). Overall, climate change has brought new challenges for crop production, with larger and more intense drought and wet seasons adding to intrinsic characteristics such as rapid carbon and nutrient turnover. These last conditions have raised new concerns such as the seeking of more technically and economically suitable alternatives to increase their production while limiting the use of potentially contaminant chemicals such as inorganic fertilizers.

Specifically, the success of crop production depends on different factors, but can be generalized as *1*) environmental conditions, *2*) quality of the seeds, and *3*) soil characteristics. Environmental conditions, above all, will define the kind of crops that can be produced in certain regions of the world. For instance, tropical soils are known to have a fast turnover of carbon and nutrients due to the typically higher environmental temperature (Vázquez-Luna *et al.*, 2019; Fujii *et al.*, 2021). Several authors such as Craswell and Lefroy (2001), Juo and Franzluebbers (2003), and Hinsinger et al. (2006) have attributed this behavior to the high demand for resources not only by plants but also by the microorganisms found within the rhizosphere. On the other hand, seed fertility and other characteristics such as resistance to diseases or extreme temperatures are normally controlled by the purchase of these from certified sources. Furthermore, soil characteristics have been historically modified by many agricultural practices to improve overall production. Regarding this, there are a known number of soil traits considered key for crop production, nonetheless, the most common one is soil fertility (van Zwieten, 2018). Moreover, as human knowledge about crop production has evolved, additional factors have arisen as important parameters that can lead to a more successful yield production. For example, the relevance of soil organic matter (SOM) not only as a source of soil structure, but also by having a key role in carbon sequestration, nitrogen mineralization, and as a promoter of plant health (Craswell and Lefroy, 2001; Hoffland *et al.*, 2020), this being especially relevant for tropical soils (Craswell and Lefroy, 2001).

As human concerns regarding environmental health have increased, the search for cleaner and safer approaches to increase crop production has also grown. Consequently, one of the explored alternatives has been the substitution of chemicals as fertilizers for safer alternatives for both human and environmental health (Eid *et al.*, 2020). Among these alternatives, the use of biomass for the promotion of plant growth has been explored by different authors (Lehmann *et al.*, 1998; Abera *et al.*, 2012; van Zwieten, 2018; Hammerschmiedt *et al.*, 2022; Jos *et al.*, 2024; Rahman *et al.*, 2024). Plant biomass has been acknowledged as an efficient soil amendment, having a significant impact on different characteristics such as (SOM) content and characteristics, as well as C and N content and dynamics (Lehmann *et al.*, 1998; Abera *et al.*, 2012) and microbial activity and biodiversity, to name a few. Nevertheless, little is known regarding the impact that biomass from specific plant families or species will have as soil amendments on final crop production. This is especially important, considering that not all plants will be able to grow worldwide. For instance, legumes are widely distributed and well known to actively interact with soil traits such as N and P cycling and microbial activity, among other important soil processes (Vázquez-Luna *et al.*, 2019). More importantly, the Fabaceae (legume) family is typically consumed by most cultures within their normal human diet as well as feedstock. Further to this, other desirable attributes for soil amendments can also be found within species of this family: fast-growing, low-maintenance, and low-cost (Hammerschmiedt *et al.*, 2022). Considering this, this study aimed to evaluate the impact of the use of biomass from three typically grown legumes on soil characteristics and the production of barley under greenhouse conditions. Results from this study would allow for a better understanding of how plant species have an influence when used as a soil amendment and their potential use as an alternative to commercial fertilizers for more sustainable cereal production.

MATERIALS AND METHODS

Soil collection and characterization

Topsoil (30 cm) was collected from an agricultural field in the central area of Mexico following the removal of the superficial layer (first 5 cm). Sampling points were selected based on local regulatory recommendations (Diario Oficial de la Federación, 2002), and following a randomized design. For this, a start-point from one of the edges of the parcel was selected, to follow a zig-zag pattern allowing a 1 m distance between sampling points. After collection, soil (400 kg) was placed into 20 kg plastic bags and properly labeled before transportation to the laboratory for handling and analysis. Once in the laboratory, the soil was spread on aluminum sheets and allowed to air-dry to be then sieved (< 2 mm) before characterization.

Soil characterization was performed following the methods recommended by local regulations (Diario Oficial de la Federación, 2002), and all chemicals were reagent grade (J.T.Baker) unless stated otherwise. Texture was assessed by the Bouyoucos method. For this, sieved soil (50 g) was placed into a 250 mL beaker and mixed with 10 mL dispersant solution and 100 mL dH_2O. The sample was then manually dispersed for about 5 minutes and qualitatively transferred into a 1000 mL measuring cylinder. Sample was then shaken over 40 s and a Bouyoucos hygrometer was finally used to assess density and temperature. Assessment was repeated after two hours based on literature recommendations. The dispersant solution consisted of 3.8 g $(NaPO_3)_6$ and 0.794 g Na_2CO_3 dissolved using 30 mL dH_2O. The proportion of each texture was calculated and the final texture was then defined. pH and electrical conductivity (EC) were assessed by a potentiometric method. For this, 10 g sieved soil was mixed with dH2O to achieve a proportion 1:3 (v:v) soil:water. Soil solution was manually stirred over 5 minutes and then assessed through a HORIBA LAQUA twin B-712 and a HORIBA LAQUA twin EC-33 for pH and conductivity, respectively.

Cation exchange capacity (CEC) was assessed by the 1N ammonium acetate method. For this, sieved soil (2 g) was placed into a 50 mL centrifuge tube to then add 25 mL solution A. The sample was manually stirred over 2 minutes and centrifuged over 5 minutes (3,000 rpm). The supernatant was discarded and 25 mL from solution B was added and stirred over 2 minutes. The centrifugation process was repeated and the supernatant was reserved for further analysis. 10 mL of the resulting supernatant from the centrifugation of solution B was transferred into a 250 mL Erlenmeyer flask and diluted with 100 mL dH_2O. Then, 10 mL buffer solution and 6 drops of Eriochrome Black T were added. The final solution was then titrated with EDTA (0.05 N). CEC was finally calculated based on the recommendations made by (Yúfera and Dorrién, 1987). Solutions A, B, and buffer were prepared as follows: Solution A consisted of 62 g $BaCL_2$•$2H_2O$ and 500 mL dH_2O, following this, 25 mL $C_6H_{15}NO_3$ was added and pH was then adjusted to 8.1 using HCl 1N. The solution was then made up to 1 L. To prepare solution B, 12.5 g $MgSO_4$•$7H_2O$ was dissolved and volume was made up to 1 L. In the case of the buffer solution, this consisted of 30 mL NH_4Cl 1N and 150 mL NH_4OH 1N.

Soil organic matter (SOM) and organic carbon (SOC) were assessed by the Walkley-Black method. In short, 2 g sieved soil was placed into a 250 mL Erlenmeyer, then 5 mL $K_2Cr_2O_7$ (1 N) solution was added followed by 10 mL concentrated H_2SO_4. The sample was then allowed to rest for over 30 minutes. Once this time has passed, 100 mL dH2O, 5 mL H_3PO_4, and 5 drops $C_{12}H_{11}N$ were added. Samples were then titrated using a $FeSO_4$•$7H_2O$ (0.5 N). Bulk density was evaluated based on the recommendations made by Diario Oficial de la Federación (2002). A tared 10 mL measuring cylinder was filled with sieved soil; the soil was allowed to settle by tapping on the cylinder to add the missing volume. Finally, the total mass of the soil was assessed; bulk density values were evaluated by dividing the mass of the soil by the volume of the measuring cylinder. Total nitrogen was obtained by the Kjeldahl method according to the recommendations made by (Fernández Linares *et al.*, 2006). For this, sieved and ball-milled soil (2 g) was placed into a Kjeldahl flask, 2 g catalyst, and 5 mL concentrated H_2SO_4. The catalyst consisted of 10 g K_2SO_4 and 1 g $CUSO_4$•$5H_2O$. Flasks were then placed into a digester and gradually heated up to 400 °C. The digestion process ended when the sample turned blue. Once the flasks cooled down, 25 mL dH_2O was added and manually shaken in order to dissolve the digested soil. Following this, the sample was qualitatively transferred into a 500 mL Erlenmeyer Flask where 15 mL NaOH (30 %) was then distilled. The distilled sample was collected in an Erlenmeyer flask already containing a solution of H_3BO_3 and an indicator. The sample was finally titrated using H_2SO_4 (0.01N). H_3BO_3 was prepared by dissolving 30 g of this substance with 750 mL dH_2O. Once dissolved, 20 mL of a mix of indicators was added (0.099 g bromocresol green and 0.066 g methyl red dissolved in ethanol (96%)).

Experimental design and selection of soil amendments

Plant species used as soil amendments were selected based on both morphological and physiological characteristics, including root length, growing time, and nitrogen fixation capacity (Lehmann *et al.*, 1998). Based on these, seven treatments and one control were selected (Table 1) using three legume species: *Medicago sativa* L.(alfalfa), *Glycine max* L. (soybean), and *Phaseolus vulgaris* L. (bean). Before growth assays, seed germination potential and viability were assessed. For this, the methodology recommended by Ma et al. (2017) was followed. In short, 10 seeds were randomly selected from each one of the treatments. Seeds were then evenly distributed and placed on a layer (5 cm) of cotton wool and into petri dishes. Petri dishes were then irrigated with a small amount of tap water and incubated at 25 °C over 48 h. After this time, the percentage of germinated seeds was estimated. In all cases, seed germination was 98% or better.

Once seed viability was assured, and to test the individual effects of each of the species biomass, seven treatments (n = 20) and one control (**Table 1**) were set up using a fully randomized arrangement. Each experimental unit consisted of a pot containing a bottom layer (2 cm) of fine gravel to prevent soil loss and 4 kg of the previously air-dried and sieved soil. Seeds were sown in each pot using 30 g when only one species was used, 15 g when two different species were tested, and 10 g for the treatment where the tree plant species were used. Seeds were allowed to germinate and grow for up to 55 days in a greenhouse (24 ± 5°C) under an 18/6 day/night photoperiod, adding water as needed. Following this time, plants were harvested and prepared as soil amendments.

Table 1. Summary of soil amendments used by treatment

Name of the treatment	Soil amendment
Control	Without amendment
T1	Bean
T2	Soybean
T3	Alfalfa
T4	Bean + Soybean
T5	Bean + Alfalfa
T6	Alfalfa + Soybean
T7	Bean + Soybean + Alfalfa

Impact of soil amendments on soil fertility and production of barley

Once plants (alfalfa, soybean, and bean) were fully grown (flower appearance), these were harvested (including roots), cut into small pieces (< 2 cm), and then mixed into the same pot with the soil that was used for their growing. Soil mixed with the harvested and cut plants was then allowed to rest over 40 days before the sowing of barley under the previously mentioned environmental conditions to promote their biodegradation. Following this time, barley was sown into each of the experimental units.

During the barley growing stage, experimental units were only irrigated using 250 mL of water once a week in order to minimize differences due to irrigation procedures. In addition, the growth test was assessed every three weeks by recording any visual detrimental changes. At the end of the growing period (120 d), the general health of the plants was evaluated through total length, number of spikes per plant, number of grains per spike, length of the spike, weight of seeds per plant, and weight of seeds per pot.

Statistical analysis

Statistical analyses were carried out using the PSPP (2023) software and a 95% confidence interval. The normality of the data was assessed by Shapiro-Wilk tests, and transformations were applied for cases where normal distribution was not presented. Significant differences between treatments were analyzed using the One-Way ANOVA test (Tukey). Graphical representations of the data were done using Microsoft Excel.

RESULTS AND DISCUSSION

Soil fertility

Initial soil characterization showed that the texture of the soil was sandy clay loam, with a CEC of 19.38 Cmol/kg and 6.9 pH. Following soil amendments and growing time, some soil characteristics (**Table 2**) were observed to be significantly modified by the treatments. Specifically, both SOM and SOC (**Figure 1**) were significantly different ($p < 0.05$) depending on the treatments.

Table 2. Soil characteristics following biomass amendments. ± represents standard deviation (n = 3). Different letters indicate significant differences between the treatments assessed by post hoc Tukey tests.

Treatment	pH	EC (µS/m)	Bulk density (g/mL)	*SOM (%)	**SOC (%)	Total nitrogen (%)
Control	6.9	268	1.25 ± 0.01^{a}	2.65 ± 0.67^{b}	1.54 ± 0.67^{b}	0.073 ± 0.004^{a}
T1	6.5	79	1.20 ± 0.01^{a}	3.02 ± 0.06^{ab}	1.75 ± 0.06^{b}	0.067 ± 0.023^{a}
T2	6.4	119	1.25 ± 0.09^{a}	2.80 ± 0.31^{ab}	1.62 ± 0.31^{ab}	0.070 ± 0.008^{a}
T3	6.6	133	1.20 ± 0.04^{a}	2.58 ± 0.18^{b}	1.50 ± 0.18^{b}	0.081 ± 0.008^{a}
T4	6.5	140	1.22 ± 0.05^{a}	2.80 ± 0.18^{ab}	1.62 ± 0.18^{ab}	0.078 ± 0.004^{a}
T5	6.4	110	1.24 ± 0.03^{a}	2.95 ± 0.12^{ab}	1.71 ± 0.12^{ab}	0.073 ± 0.012^{a}
T6	6.2	145	1.25 ± 0.04^{a}	3.32 ± 0.06^{a}	1.92 ± 0.06^{a}	0.089 ± 0.004^{a}
T7	6.4	63	1.22 ± 0.02^{a}	2.57 ± 0.12^{b}	1.51 ± 0.12^{b}	0.065 ± 0.004^{a}

*SOM = Soil organic matter, **SOC = Soil organic carbon.

For both cases, treatment 3 (soil amended only with alfalfa) had significantly smaller amounts of organic matter and organic carbon than the control. On the contrary, treatment 6 (soil amended with alfalfa and soybean) was significantly higher than the control. As for the other parameters, results showed that the soil amended with biomass from alfalfa and soybean consistently modified soil characteristics, reducing pH, EC, Ca^{2+}, and K^{+}, while increasing particle density, total nitrogen, and NO_3. Regarding soil quality and its impact on crop production, characteristics such as SOC have been established to have a beneficial effect on plant nutrition (van Zwieten, 2018). Among these favorable impacts are larger amounts of mineralizable N and P and better structural properties such as lower bulk density and better aggregate stability (van Zwieten, 2018).

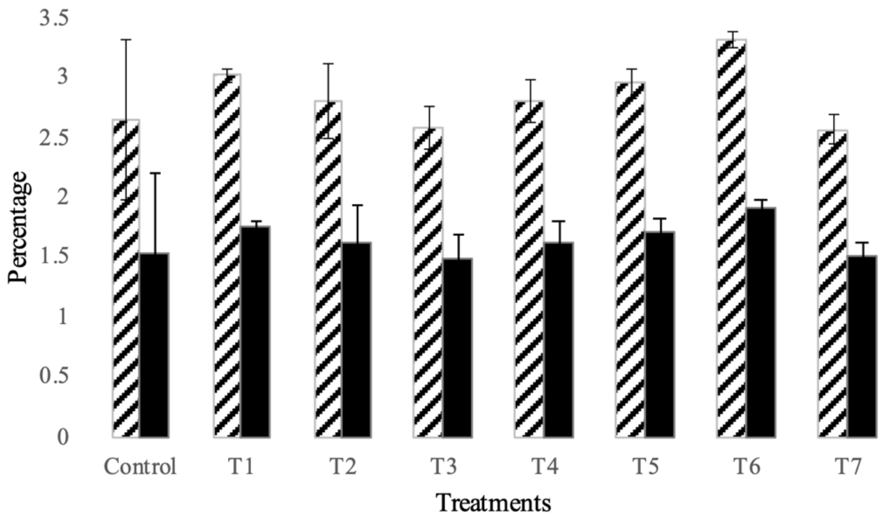

Figure 1. Soil Organic Matter (▨) and Soil Organic Carbon (■) of soil after 15 weeks of interaction of the amendments with soil and growth of barley. Error bars represent standard deviation.

As for the case of this study, results showed significantly larger amounts of both SOM and SOC, although this was not reflected in the soil total N, where significant differences were not observed when compared against the control. It is important to mention that only one of the treatments produced significant differences when biomass from alfalfa and soybean was mixed and incorporated into the soil (Chivenge *et al.*, 2011; van Zwieten, 2018). One of the reasons why total N was not increased by the treatment, in accordance with the stated by van Zwieten (2018), might be that even though the experiment was run over 15 weeks, this time was not enough to have an impact in soil fertility, in this case, reflected as total N. This is supported by the reports made by different authors, where the long-term impact of using organic amendments is noticed only after several years of application (Eldridge *et al.*, 2018; Allam *et al.*, 2022). Moreover, this effect is even more noticeable when a combination of both inorganic fertilizers and organic amendments is used followed by a long period of application (Goyal *et al.*, 1999). These reports range from five up to 15 years of application (Goyal *et al.*, 1999; Mangalassery *et al.*, 2019). Interestingly, these cases have also pointed the relevance of organic amendments not only from the nutritional aspect but also considering the enhancement of soil structure given by the organic matter from the amendments. This aspect is especially important in tropical and subtropical soils used for agriculture, often characterized by poor structure, and low organic carbon and nutrient availability (Prado *et al.*, 2016; Ahirwal *et al.*, 2021).

Another condition that might have an influence towards total N in the present study might be due to the role of the amendment (plant biomass) as a sorbent. This effect, especially at the initial stages of biodegradation of biomass might be reflected as no difference in nutrient concentrations or even as a reduction of both macro and micro-nutrients. A similar behavior has been reported in studies from different areas of soil science, especially by those studying the control of contamination in soil (Dubey *et al.*, 2014; Vázquez-Cuevas *et al.*, 2018). By this, plant material at early stages of decomposition will sorb bioavailable nutrients to be then gradually desorbed as a consequence of natural biochemical processes, especially soil pH and microbial activity. Additionally, something important to note regarding the results from this study is the impact that all treatments had on lowering soil pH. Although this is the opposite of what has been reported as the common behavior in similar studies (van Zwieten, 2018), this can be attributed to specific interactions between the amendments and the soil's physical and biological characteristics. Specifically, this behavior has been previously observed when root biomass is used as an amendment, due to the large amounts of organic acids that roots commonly contain and exudate (Jones and Darrah, 1994). Moreover, it has been proposed that during root decay and turnover, organic acids such as citric and malic acid are released into the soil (Jones and Darrah, 1994; Vázquez-Cuevas *et al.*, 2020), hence producing lower pH values and inducing larger microbial communities. This process has been reported as a common mechanism in plants to promote the desorption of macro and micro-nutrients to be then incorporated as nutrients by plants (Hinsinger *et al.*, 2006). This is commonly known as the rhizosphere effect (Kuzyakov, 2002).

Furthermore, it is important to note that although fertility (assessed as total N) was not affected by the treatments, this was not the case for SOM and SOC. This finding gains major importance when its ecological significance is considered. SOC has been consistently known as an indicator of soil quality (Kumar *et al.*, 2018), as it is associated with more productive areas. Nonetheless, soil carbon storage goes further, and it is nowadays recognized as a key process within climate change mitigation (Sommer and Bossio, 2014). Moreover, environmentally mindful practices such as using organic amendments instead of inorganic fertilizers have been constantly studied. Among a range of advantages, using biomass amendments could potentially promote carbon sequestration up to SOC's equilibrium point (Sommer and Bossio, 2014), avoiding its normally observed depletion in agricultural soil (Kumar *et al.*, 2018). Regarding legumes, this family has been reported to significantly enhance C sequestration when compared to other crops (Kumar *et al.*, 2018). Results from this study support the relevance of using legumes to promote SOC sequestration. Further to this, although it was not within the aims of the present study, authors have hypothesized that this might be due to specific legume-soil-bacteria dynamics. For instance, (Hu *et al.*, 2024) attributed higher SOC in the presence of legumes to a plant-microbe induced availability of carbon and nitrogen forms.

Production of barley

Soil amendments were observed to produce significant differences in the overall growth and production of barley. In this case, all treatments produced significantly larger ($p < 0.05$) spikes (**Figure 2**) as well as a significantly higher number of grains per spike ($p < 0.05$) (**Figure 3**). In this case, and as for their influence on SOM and SOC, treatment 6 (amendment using alfalfa and soybean biomass), was the one resulting in a better performance.

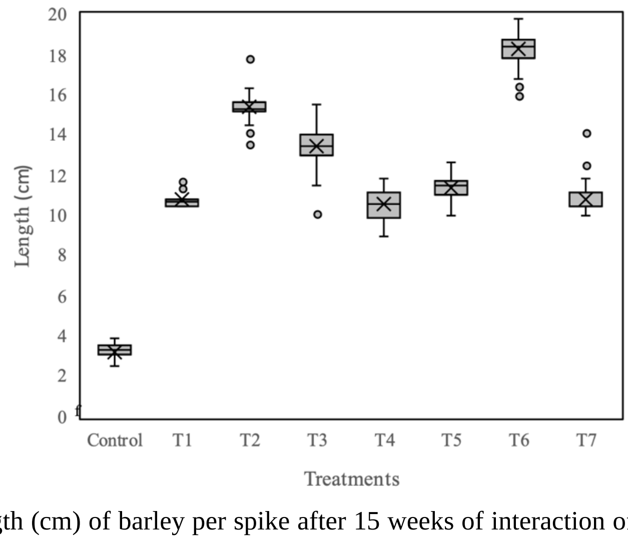

Figure 2. Mean spike length (cm) of barley per spike after 15 weeks of interaction of the amendments with soil and growth of barley. Error bars represent standard deviation.

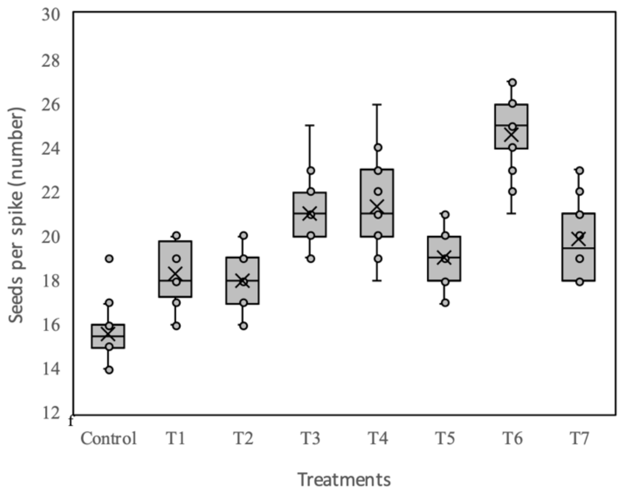

Figure 3. Mean grain production of barley per spike after 15 weeks of interaction of the amendments with soil and growth of barley. Error bars represent standard deviation.

Similar results were also observed when overall growth was analyzed. This is reflected by faster growth and a bigger total length of the plants over the growing time (15 weeks) as can be observed in **Figure 4**. The rest of the treatments had similar behaviors among them, exhibiting significantly better performances than the unamended control. Regarding the impact of the studied amendments on the production of barley, overall results showed that all treatments increased both the length of the plants and the number of grains per spike. Historically, the use of organic amendments has shown to have a potentially significant role in soil characteristics and ultimately crop production, showing in most cases larger productivity than non-amended soil (Román-Gutiérrez *et al.*, 2019; Allam *et al.*, 2022). This statement is in complete accordance with the results of the present study, where all treatments were shown to significantly increase plant physical attributes.

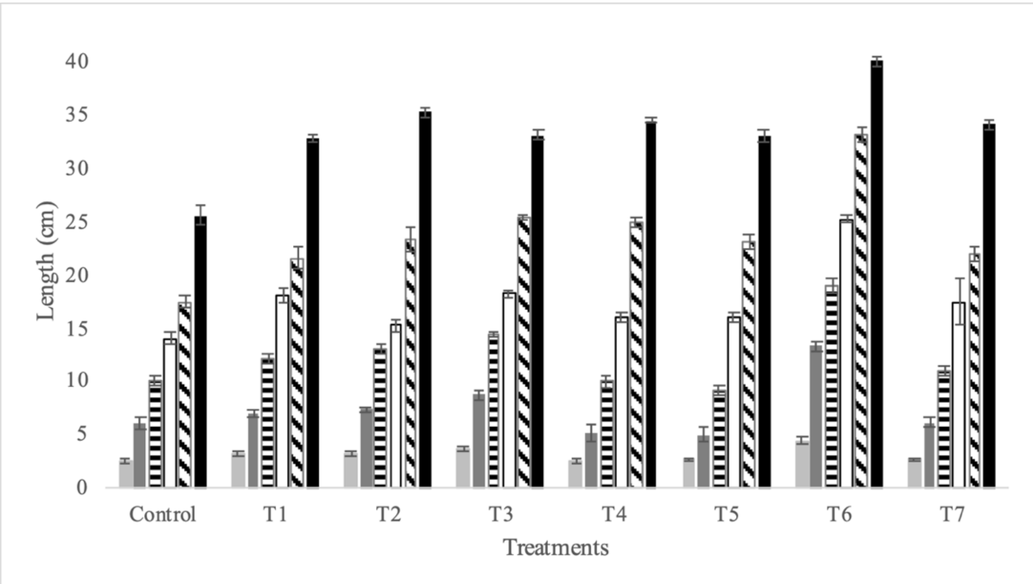

Figure 4. Overall growth of plants after 1 (☐), 3 (■), 6 (▤), 9 (□), 12 (▧) and 15 (■) weeks. Error bars represent standard deviation.

Nonetheless, the reason why this is a common result of organic amendments has not been completely understood yet. In this case, plant biomass was used as a soil amendment. However, the most common organic amendment is compost (Vu, 2023), followed by other organic materials such as manure (Eldridge *et al.*, 2018) and sewage sludge (Eid *et al.*, 2020). Despite this, the overall benefits of the use of organic amendments can be generalized as a direct supply of macro and micro-nutrients, especially regarding nitrogen. Even though these benefits are consistently reported, it has also been acknowledged that the quality and quantity of these benefits will differ based on the type and characteristics of the biomass (van Zwieten, 2018; Román-Gutiérrez *et al.*, 2019; Vu, 2023). The results of this study are under this last statement, showing a significantly larger production of barley when a specific treatment was applied, in this case, the combination of alfalfa and soybean biomass.

Although the specific reason for this behavior is still under debate, the overall consensus is the distinctive characteristics of each species and even the age of the plants will ultimately define how plant biomass will impact both soil and grain production (Pržulj and Tunguz, 2022). As the general composition of plants consists mainly of cellulose, hemicellulose, and lignin (Pržulj and Tunguz, 2022), the amount of these will greatly influence its rate and specific turnover mechanisms (Walela *et al.*, 2014; Baitsaid *et al.*, 2018). Specifically, when looking at specific nutritional contents of both alfalfa and soybean, some differences suggest that the combination of these two plants as soil amendments might offer a complete range of macro and micro-nutrients and ultimately produce a larger crop production. This considering that the alfalfa plant is recognized to have a high content of nitrogen (Pop *et al.*, 2010), while the soybean plant contains a wide range of micro-nutrients (Kahraman, 2017). Moreover, alfalfa has been previously reported to significantly enhance soil fertility, leading to its proposal as a tool to restore degraded soils (Mei *et al.*, 2024), while soybean has been acknowledged to have a more efficient P usage process than other legumes (Pang *et al.*, 2018). Further to this, another reported reason for significantly enhanced plant growth following organic amendments is the one observed by Li et al. (2021) and Zhao et al. (2020), concluding that this effect was due to the impact that the plant residue had on soil bacterial community and the labile fraction of carbon, ultimately improving soil fertility.

Despite the aforementioned, it is important to note that according to several reports, long-term benefits from the use of plant biomass as an organic amendment might only be reached if used in combination with inorganic fertilizers (van Zwieten, 2018). Although this might look like going in the opposite direction of what would be desired, the combination of organic and inorganic fertilizers could represent a more viable alternative. Nowadays, one of the most common reasons for not using organic fertilizers within commercial crop production is the uncertainty that this practice represents when compared to traditional practices. Moreover, by combining the use of these two types of soil amendments, producers might have a better idea of their production and still reduce some of the more harmful consequences of using inorganic fertilizers, such as the emission of greenhouse gases and soil contamination. This, and the development of proper protocols might represent the next step in the study of alternatives for better and safer crop production.

CONCLUSION

In this study, soil amendments with a combination of alfalfa and soybean had a significant impact increasing SOC (+ 0.38%) and SOM (+ 0.65%) when compared against the control. Nonetheless, this was not reflected in a higher fertility (as total N). Results also showed that short-term use of plant biomass (up to 15 weeks) might cause lower soil pH values, possibly attributed to the release of organic acids from the plant root material into the soil. Regarding plant species, the specific use of plant material from alfalfa and soybean significantly enhanced the production of barley, including both plant biomass and the number of grains per spike when compared to the control. This effect can be attributed to the combination of chemical characteristics from both species, providing the necessary macro- and micronutrients to promote barley growth and grain production. Overall, the mixture of alfalfa (*Medicago sativa*) and soybean (*Glycine max*) as soil amendment produced both a better soil quality and a higher barley production. Observations from this study provide relevant data regarding the feasibility of improving crop production using organic amendments However some challenges must be addressed to apply these at a larger scale. First of all, specific soil requirements should be established, and a trial has to be set up to prior assess its efficiency. Moreover, by doing this, a predictable and manageable protocol could be designed. Moreover, this practice might represent a sustainable strategy to prevent soil degradation by the incorporation of SOC and SOM into the soil, being this especially important in tropical and subtropical agricultural soil. Additionally, an estimation of overall costs should be performed on a site-specific basis, due to the high variability that agricultural practices will have based on environmental factors and technology adoption.

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