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Soil column-based experimental design to assess the impact of compost and vermicompost amendments on maize biomass

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Abstract

Most experiments measuring the positive impact of organic matter (OM) amendments on plant water stress were conducted in pots and did not take into account the vertical extension of the plant root system. Our goal was to build an experimental setup able to mimic the geometry of organic amendments distribution relative to plants, i.e. amendments mixed with the top layer (0-10 cm) when most of the roots develop below this layer. We had 2 objectives (i) to test our soil water monitoring instruments and (ii) to check if a promoting effect of OM amendments on the growth of maize (*Zea mays* L.) plants was possible in soil columns. The PVC columns (60 cm high, 20 cm diameter) were filled with a loamy soil; the organic amendments were mixed with the top layer at a rate of 20 t/ha. We controlled 3 variables: the type of organic amendments (compost vs vermicompost), fertilizer amount (high/low amount) and soil compaction (high/low). Water stress was induced by gradually letting the soil dry to 50 % of the field capacity. Plant growth and soil water status were measured simultaneously during 6 weeks; at the end of experiment above and below ground biomass were measured. In the presence compared to the absence of OM, the plant height and plant above ground biomass were significantly higher, approximately multiplied by 2. Despite similar soil porosity in control and vermicompost columns, the latter had significantly our technical experiment has shown that the characteristics of our columns and our monitoring instruments were able to record changes in soil water content that could be associated to difference in plant development. A very strong promoting effect of OM was measured, but no difference was observed between compost and vermicompost as the experimental design was made mainly for technical purpose.

Keywords: water stress, organic matter, bulk density, biomass, maize

1. Introduction

Recent experiments in paddy fields have shown that inorganic fertilizers have a more pronounced positive effect on rice biomass and yield if they are applied concomitantly with organic matter (OM) [1]. Such a result is quite surprising because OM is generally considered as amendment rather than fertilizer. This may be related to the fact that rice roots tend to preferentially develop and remain in the topsoil layers, *i.e.* just underneath the layer of OM amendments. It is unclear whether the situation would be the same in the case of crops that develop a deeper root system.

Vermicompost is known to improve soil physical and chemical properties and to promote plant growth

[2]. Results of other experiments suggest that vermicompost could also help mitigate water stress more efficiently than compost [3]. In the lowland Laos, where the climate is characterized by a long dry season, such an effect, if averred, would represent a major advance for minimizing crop damage and loss from drought. Yet, experimental conditions under which such an effect was detected do not compare with field conditions to the extent that the whole root system of plants was placed in direct contact with the applied organic matter. In contrast, in the field, OM can only be mixed with the upper soil layers (*i.e.* the top 10 to 20 cm layers at most), meaning that a large portion of the root system is not in direct contact with it.

Most experiments measuring the positive impact of organic matter (OM) amendments on plant water stress were conducted in pots and did not take into account the vertical extension of the plant root system. To test whether OM application, namely compost and vermicompost, can have a plant growth promoting effect on a real soil profile, we carried out a soil column experiment. The goal of this column experiment was to mimic the geometry of OM distribution relative to plants root system in field situations, i.e. amendments mixed with the top layer (0-10 cm) when most of the roots develop below this layer. Monitoring water movement in soil columns at short time scale (every minute to every hour) is not an easy task and needs adapted instruments and the skill to run them and collect and process the data during several weeks. Failures and mistakes can easily happen, meaning that a lot of work and time has to be spent again on preparing the soil columns. Thus we wanted to conduct a preliminary experiment to test the different instruments and the reaction of a test plant to OM amendments are the skills of that kind of monitoring do not really exist in Laos and Thailand.

In our preliminary experiment, we had two main objectives (i) to test our soil water monitoring instruments and (ii) to check if a promoting effect of OM amendments on the growth of maize (*Zea mays* L.) plants was possible in soil columns. In this paper we present the main features of this experimental design and briefly discuss the results of a preliminary experiment based on this design.

2. Material and methods

2.1. Soil characteristics

Soil was collected on the campus of the Faculty of Agriculture of the National University of Laos, located at Nabong, 30 km Northeastern part of Vientiane. After removing the surface layer to avoid including organic debris, soil from depths ranging from 10 to 30 cm was collected. This soil had very low nutrient content and is therefore of marginal value for agriculture; its main characteristics are presented (Table 1). The soil water content was 12% (mass-based) at the time of collection; it was kept at this moisture content and sieved (3 cm mesh) to have a more homogeneous structure when used to fill PVC columns.

2.2. Experimental design

We tested the effect of 3 factors:

- Initial soil porosity: sieved soil was repacked at initial porosity levels of 0.392 and 0.290 cm^3/g, referred to as high porosity (H_p) and low porosity (L_p), corresponding to soil bulk densities of 1.3 and 1.5 g/cm^3, respectively;
- Amount of fertilizers: fertilizers were applied at 2 rates referred to as high and low fertilisation. High fertilisation (H_f) consisted of 15-15-15 (N/P/K) at 2.9 g/plant and 46-0-0 at 2.3 g/plant; low fertilisation (L_f) consisted of 15-15-15 at 0.5 g/plant and 46-0-0 at 0.4 g/plant. Note that 15-15-15 fertilizer was mixed to the soil before seedling planting and 46-0-0 fertilizer was applied to the soil surface after planting at the time of irrigation, on 22 July and 11 August 2015.
- Type of organic amendment: compost (C) and vermicompost (V) were prepared from the same mixture of cow dung and coconut fibre (Table 2), the ratio was 2:1 by volume.

Table 1 Main soil characteristics

Parameters	Values
clay	22%
silt	31%
sand	47%
Soil texture	Loam
pH (H_2O)	4.16
pH (KCl)	4.09
OM (%)	0.80
Total N (%)	0.04
Available P (mg/kg)	2.24
Exchangeable K (mg/kg)	11.53

Table 2 Main characteristics of the compost and vermicompost

Parameters	Materials	
	Compost	Vermicompost
pH(H_2O)	7.11 ± 0.12	6.56 ±0.32
C/N Ratio	19.60 ± 0.97	20.10 ±1.37
OC (%)	31.22 ± 1.21	29.60 ±2.06
Total N (%)	1.60 ± 0.03	1.46 ± 0.09
Total P_2O_5 (%)	1.45 ± 0.29	1.26 ±0.21
Total K_2O (%)	0.77 ± 0.13	0.85 ±0.14
Total Na (%)	0.22 ± 0.06	0.20 ±0.05
Total CaO (%)	2.62 ± 0.18	2.34 ±0.46
Total MgO (%)	0.68 ± 0.08	0.69 ±0.18
Total S (%)	0.16 ± 0.03	0.22 ±0.06
EC (dS/m)	1.98 ± 0.31	2.76 ±0.61

Two additional columns were used as a control: no organic matter was added to those two columns and both had porosity equivalent to H_p treatments, *i.e.* 0.392 cm^3/g but they received different fertilisation

rates, one column was similar to the H_f and the other to the L_f treatments.

The experimental design and the treatments applied to the different columns are summarised in (Table 3). There was no replication of any treatment as the primary goal of this experiment was to test the feasibility of the column-based experimental design and to identify technical issues to be resolved prior to expanding to a full-scale experiment. Nevertheless, comparisons presented and discussed in this paper primarily target the role of vermicompost and compost on plant development (particularly plant height change); to this extent, there were 4 pseudo-replicates for these two types of OM treatments.

Table 3 Experimental design for the treatments applied on the soil inside the column. With (i) Porosity H and L were 0.392 and 0.290 cm^3/g respectively, Fertilizer H and L were 5.3 and 0.9 g/plant respectively; C and V were 63 g/plant or 20 t/ha of Compost and Vermicompost [2], [3], [4], [5], [6], [7] & [8]

	Columns	Porosity	Fertilizers	OM	Code names
Treatments	1	H_p	H_f	V	$H_p H_f V$
	2			C	$H_p H_f C$
	3		L_f	V	$H_p L_f V$
	4			C	$H_p L_f C$
	5	L_p	H_f	V	$L_p H_f V$
	6			C	$L_p H_f C$
	7		L_f	V	$L_p L_f V$
	8			C	$L_p L_f C$
Controls	9	H_p	H_f	0	$H_p H_f 0$
	10		L_f	0	$H_p L_f 0$

2.3. Columns preparation

Sieved soil was poured into each PVC column and repacked as 3 individual layers, each 10 cm thick, to reach a total thickness of 30 cm. Total amounts of 4.57 kg or 5.28 kg of soil per layer were used to obtain the desired bulk densities (BD) of 1.3 or 1.5 g/cm^3, respectively, corresponding to the H_p and L_p porosity treatments (Figure 1). The organic matter and 15-15-15 fertilizer were incorporated in the soil prepared for the upper layer (0 to 10 cm depth).

Once the soil repacked in a PVC column, some water was gently added to reach a total water content (Wc) of 15 % (g/g), that is closed to field capacity. Note that the volumetric water content (θ cm^3/cm^3) can be calculated as follow:

$$\theta = W_c \times BD \quad (1)$$

In only one column (L_pL_fC), 2 small windows were opened at 10 and 15 cm depth below the soil surface to insert 2 Time Domain Reflectometry (TDR) probes (TRIME-PICO32) [9]. They were connected to a data logger that recorded the volumetric water content every 10 minutes from 17 July to 14 August 2015. Note that the initial volumetric water content was:

$$\theta = 15 \times 1.5 = 0.225 \; or \; 22.5\% \quad (2)$$

Air moisture and temperature, as well as soil temperature were also monitored during the whole duration of the experiment, from 30 June to 14 August 2015, at constant time intervals of 10 minutes.

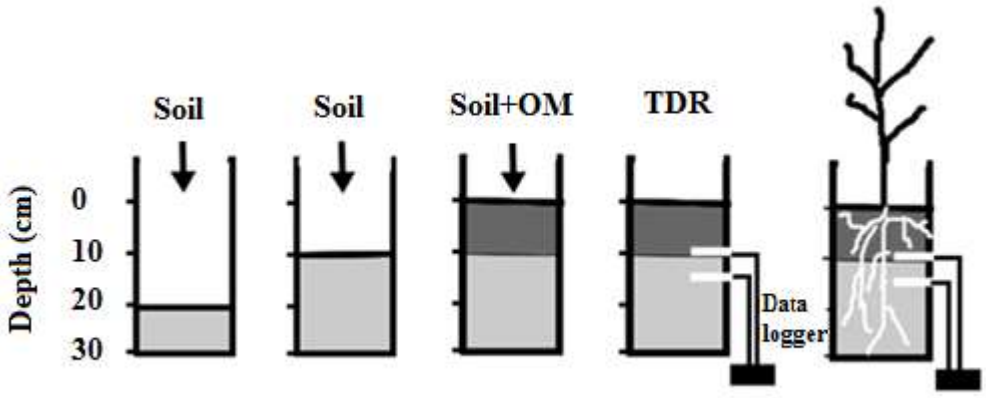

Figure 1 Mains steps of column filling, note that OM is incorporated only in the upper layer (0-10 cm depth). TDR probes were inserted at 10 and 15 cm in one column. The last drawing is a schematically presentation of maize shoots and roots.

2.4. Maize germination, planting and growth monitoring

Maize was selected as it is a common model plant used by agronomists and soil scientists, and also because it is widely grown in South-East Asia, making it easy to find varieties adapted to the local conditions. We selected the Pacific 999 super hybrid variety of (Pacific seeds Ltd., Thailand) [10]. For germination, seeds were enclosed within cotton bags and soaked in warm water (40°C) for 3 hours; after 4 days, two vigorous germinated seeds were selected and buried under 5 cm of soil, in each soil column (11 July 2015); 4 days later the most vigorous seedling was kept while the other was removed (15 July 2015); the height and number of leaves were measured daily for each maize plant,

2.5. Irrigation

Columns were weighed every day to estimate the daily water loss due to evapotranspiration. Between 30 June and 16 July 2015, we maintained the total water content at 15 % (g/g): daily water loss was compensated by irrigation made with a hand sprinkler. On 17 July, as the maize plants already had 4 leaves, we induced plant water stress by modifying irrigation according to 4 successive steps:

1. Reduction of the water content to 80% of its original value on 22 July, i.e. 18 % (v/v) in the L_pL_fC column (equipped with TDR);
2. Restoration of the original water content and subsequent reduction to 65% of the original water content on the 31 July, i.e. 15% (v/v) in the L_pL_fC column;
3. We repeated the same cycle as described in 2 and reduction to 65% of the original water content was reached on 5 August;
4. Further reduction of the water content down to 50% of the original WC reached on 11 August, i.e. 12 % (v/v) in the L_pL_fC column.

The experiment was stopped on 14 August.

2.6. Soil and plant characteristics at the end of the experiment

Maize plants of each column were harvested, separated in stem, leaves and flowers if any. They were dried at 65°C for two days and the plant dry biomass was subsequently measured.

Soil samples were collected in each layer (0-10, 10-20 and 20-30 cm) using 100 cm^3 steel rings (n = 5). Maize roots were collected out of these 100 cm^3 soil samples using tweezers. Roots and soil were dried at 65°C and 105°C respectively for 2 days.

The bulk density and the specific pore volume were calculated as follow:

$$BD\ \left(\frac{g}{cm^3}\right) = \frac{Dry\ soil\ mass}{Soil\ volume} \quad (3)$$

$$Specific\ Pore\ Volume\ \left(\frac{cm^3}{g}\right) = \frac{1}{BD} - \frac{1}{2.65} \quad (4)$$

3. Results and discussion

3.1. Plant development

Figure 2 shows that the irrigation pattern had a noticeable effect on plant growth. Daily irrigation from 11 to 16 July ensured a regular increase in plant height that was similar for all treatments until 19 July. On 20 and 21 July, plant height remained virtually unchanged in the control while it was still regularly increasing in the 2 treatments. This drastic slowdown in plant height change in the control is indicative of a water stress, which did not occur in the OM treatments. The irrigation made on 22 July allowed plant height to increase again in all columns, but only for 3 days in the control (i.e. until 25 July) while in the 2 treatments it continued to increase, although at a slower rate from 26 July onward (i.e. 4 d after irrigation). Irrigation on 31 July allowed plant height increase again, at a moderate rate in the control and at a faster rate in the treatments: the average daily plant height increase was 3 cm/day over the whole duration of the experiment, but it almost doubled between 31 July and 1 august (~ 6 cm/day). In both control and treatments plant height increased until the next irrigation. Similar rates of height increase were observed after irrigation on 5 August. Between 7 August until the last irrigation on 11 August, plant height remained unchanged in all treatments. A final phase of plant height increase, rather rapid and then weaker, was observed in all treatments following the last irrigation.

Our irrigation pattern was able to induce water stress as indicated by variations in plant height changes. Overall, water stress appears to have been more severe in control columns than in columns that received an addition of organic matter, as final plant height was approximately 90 cm in the presence of OM and only 50 cm in the control. However, there was no difference related to organic matter quality: plant height change was not significantly different for compost and vermicompost.

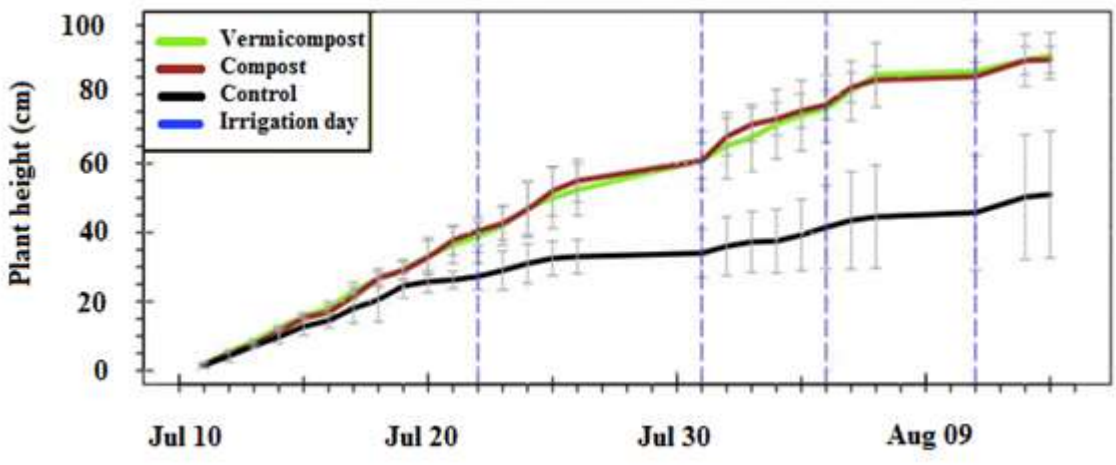

Figure 2 Daily plant height (cm) for the columns containing vermicompost (n = 4), compost (n = 4) and control without any organic matter (n = 2). Daily irrigation was made daily from the onset until 16 July (not indicated on the figure) and then only 4 times on days indicated by the vertical dotted line.

As there was no effect of OM quality but a strong effect of the presence/absence of OM on plant height change, we decided to analyse in greater detail the impact of OM on plant development depending on the fertilisation level. To this end, we compared plant biomass depending on both OM and fertilisation applications (Figure 3). Irrespective of OM application, this comparison revealed that plant biomass was proportional to the fertilisation level: with OM application, the higher fertilisation rate resulted in 30 % higher plant dry biomass (40 vs 30 g) while without OM, the difference exceeded 100 % (17 vs 5 g). However, it can also be seen from Figure 3 that OM addition had a pronounced effect on plant biomass as for both the low (Lf) and high fertilisation treatments (Hf), it induced a six- and more than two-fold biomass increase (from 5 to 30 and 17 to 40 g, respectively). This most likely indicates that OM plays a role in mitigating the negative effect of water stress on maize plant growth.

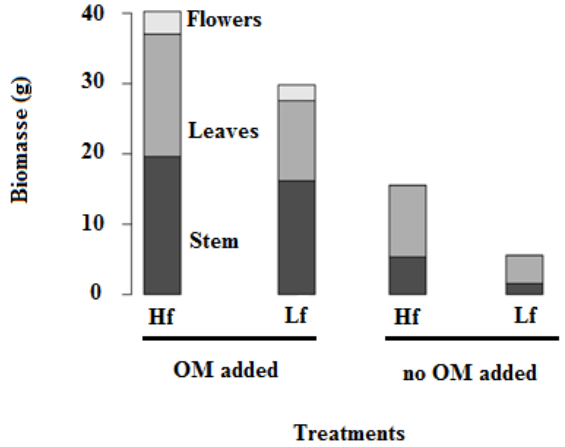

Figure 3 Stem, leaves and flower dry biomass in relation with level of fertilisation and use of organic matter. The 2 bars on the left present the average values of 4 columns (2 OM types X 2 bulk densities); each of the 2 bars on the right side present the value of a single column (no replicates).

Further, it can be noted that without OM addition, plants did not reach the flowering stage within the duration of the observation period while they did with OM addition. It is therefore possible that OM ultimately has an effect on yields and this is a point that deserves further attention

.

3.2. Soil water monitoring

One single column was equipped with TDR to record changes in volumetric water content at 10 and 15 cm below the soil surface (Figure 4). The blue line in Figure 4 represents the volumetric water content derived from daily column weight measurements, starting from the initial water content of 15 % g/g (22.5 % v/v). The TDR measurements show that water was not uniformly distributed within the column, with the upper layer displaying higher water content after irrigation than the one in the lower position, probably due to its position relative to the rewetting front during irrigation. This observation indicates that the upper soil layers probably stored most of the irrigation water. Preferential water storage in the upper layers could be related to the presence of OM but in the absence of similar measurements on control columns we do not yet have data to confirm this hypothesis.

From 17 to 31 July both probes recorded a regular decrease in water content concomitantly with a decrease in the difference in water content between the two soil depths. In addition, it can be seen that water content values derived from TDR probes were systematically lower than the water content computed from column weight measurements (blue line). While this discrepancy may reflect for part a calibration problem (it can be seen that there is an abrupt shift towards slightly lower in TDR values on 22 July, even though water content should have increased at that

time following irrigation), it must also be noted that due to the accumulation of biomass related to plant growth, it is not unexpected that the two methods yield irreconcilable values, all the more that plants grow bigger.

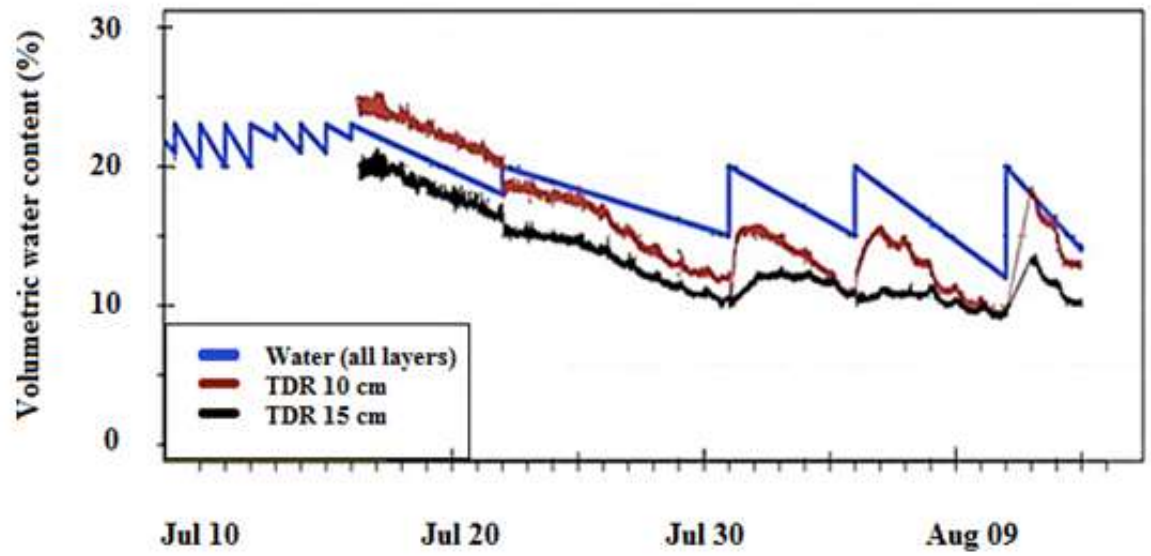

Figure 4 Variations in soil volumetric water content (%) over a one-month period: total water content derived from changes in column weight (blue line) and from TDR probes inserted at 10 and 15 cm depth (red and black lines).

3.3. Bulk density and root profiles

For the purpose of this assessment, we selected 3 columns with low fertilizer content: one control column with high porosity (HpLf0,), and two columns that had received OM addition, one with high porosity (HpLfV), the other one with low porosity, (LpLfC). In each repacked layer of each column, we collected undisturbed soil cylinders that were used to measure soil porosity and root biomass.

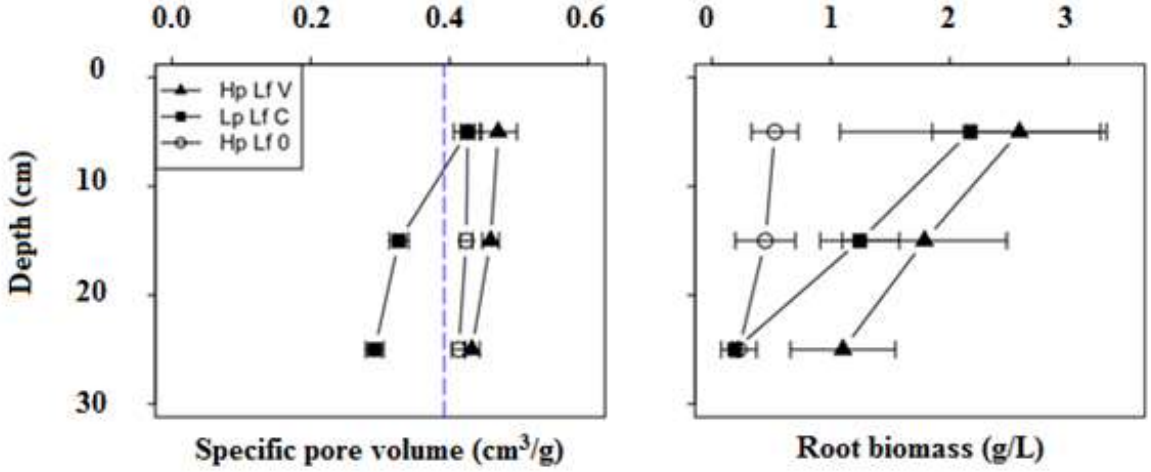

Figure 5 Left: Porosity profile; Right: root biomass measured inside the same cylinders.

Figure 5 (left) presents soil porosity profiles. The low standard deviation around the mean porosity indicates low structural variability in a given layer. Except for one case (LpLfC at 20-30 cm), the pore volume measured at the end of the experiment was higher than the porosity we created at the beginning of the experiment. The increase was higher in the top than in the bottom layer and it was ranging from 0.08 to 0.04 cm^3/g for HpLfV and 0.03 to 0.02 for HpLf0. One factor that can possibly explain this increase in soil porosity, at least for part, is root development. However, porosity increased up to 20% (0.08 vs 0.39), which is quite substantial. Considering that the maximum root density is of the order of 3g/L (Figure 5, right) and if we assume a specific root length of 10 m/g and a mean root diameter of 1 mm, this leads an

increase in porosity of the order of only 2.5%. Thus, root growth alone unlikely explains all the observed additional porosity; neither does the presence of OM, as a porosity increase was also observed in the control columns were no OM was added. At this stage of our experiment, the origin of the porosity increase remains unexplained and complementary measurements must be conducted to unravel the process at the origin of such a structural change.

Despite this inconsistency, it must be noted that the pore volume was of the order of magnitude that we intended to create at the beginning of the experiment with very high homogeneity in all layers and across treatments. Unlike porosity, root content displayed a large variability inside a given layer and between treatments. In addition, we observed a decrease in root content with depth with values > 2 g/L when OM was incorporated in the top layer and of only 0.53 g/L in the absence of OM. With OM addition, in the second layer, the root content remained > 1 g/L and was still significantly higher than in the control (0.47 g/L). In the bottom layer, root biomass > 1 g/L in the HpLfV treatment only, which was significantly higher than in the 2 other columns that had a similar root density of 0.2 g/L. The much higher root densities in the column containing OM likely explains why water stress always occurred later in this treatment than in control columns: with more roots, plants were able to utilize more efficiently the soil water stock. With very high root densities in the top soil, they also more used water more readily than plants in columns that did not receive OM, which might explain why most of the irrigation water remained stored in the top layer and did not recharge the underlying layer, as suggested by (Figure 4).

It is noteworthy that there was no relation between total pore volume and root development: the control column had a high porosity but the smallest root biomass. As root development is sensitive to total pore volume and soil mechanical resistance to penetration, one would expect that adding OM could significantly reduce the mechanical resistance, favouring the root development at early stages of plant growth. But OM was only added to the top layer and higher root biomass was also observed in the second layer; this indicates that benefits from OM addition were probably not limited to the soil layer in which OM was added.

4. Conclusion

Our objectives were to test whether OM application in the top layer can have a plant growth promoting effect on a plant grown in a soil column experiment with root growing also in deeper layers that do not contain OM and discuss the validity of our experimental design and the results about OM impact on soil and plant.

We have validated the use of a soil column and the way to prepare the different layers as (i) roots were observed not only in the layer containing the OM but in all the 30 cm column; (ii) no structural or functional discontinuities were observed between the layers and (iii) no general soil collapse was observed, indicating a sufficient soil structural stability when irrigation was gently made.

The monitoring of column weight and TDR measurement provided useful information on the water location. But water content is only a proxy of water stress; more accurate data are needed on the water potential that is the physical characteristic to which plant is sensitive. From TDR we know that irrigation water mainly remains in the top layer, suggesting that it is the layer where tensiometers should be installed as the other layers will probably have lower water content and thus lower matric potential.

Concerning the treatments, we observed that:

- The porosity 2 levels did not have any impact on plant development
- Fertilizers: it is a strong limiting factor in the poor soil are using for this experiment.
- Type of OM: C and V had the same composition and no difference was observed on plant shoot development, even if differences were observe on the root system; but it was not conclusive as the porosity differed at the same time as the type of OM and we did not have replicates to make a statistical analysis of our results.

Thus, in the future experiment any of the porosity level can be selected but fertilisation must be done only at high rate for the nutrient content not to be a limiting factor. About OM, complementary analysis should be done about their biochemical characteristics (presence of growth hormones, etc…) and more detailed measurement should concerning the root characteristics.

The plant water stress was measured with a very global indicator (increase in plant height); a characteristic more precisely related to photosynthesis and water stress should be measured.

Moreover, we have measured a very high impact of OM compared to the treatment without OM. But the experimental design cannot discriminate the factors and processes explaining this difference that can putatively be attributed to chemical, biochemical or physical characteristics. Thus, we could use columns with high level of fertilisation and control columns containing inert OM like coconut fibber, as it would not provide any mineral nutrient or hormone to the plant, but it would impact only the physical characteristics of the top layer. Using coconut in the control, we could check if it is worth to prepare compost or vermicompost, or otherwise, if putting any other organic matter is also efficient.

Finally, we can conclude that our experiment has confirmed the strong effect of OM addition, even if we are not able to conclude on a specific benefit from vermicompost compared to compost.

5. Acknowledgements

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