Preliminary results of a pot experiment with the combined effects of a terrestrial isopod species (*Porcellionides pruinosus*, Brandt 1833) and organic mulching on tomato

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Abstract: Organic mulching practice has a beneficial effect on soil life. Increased soil activity may in turn lead to increased soil fertility. To study the effect of terrestrial isopods and organic mulch on potted tomatoes we measured the growth, vitality and yield of plants. Half of the pots had the surface of the soil covered with a mulch of equal quantity of walnut (*Juglans regia*), elm (*Ulmus minor*) and maple (*Acer platanoides*) leaves; and half of them had the soil uncovered. 20 *Porcellionides pruinosus* (Brandt, 1833) individuals were introduced to every second pot. There were four treatments in 10 replicates: (1) I+M+ (isopods, mulching); (2) I+M- (isopods, no mulching); (3) I-M+ (no isopods, mulching) and (4) I-M- (no isopods, no mulching). To determine the microbial activity of the soil, fluorescein diacetate hydrolysis activity was measured. The fluorescein diacetate concentration was higher in the “combined” treatment than in the “isopods only” treatment, and in the “mulch only” treatment. Regardless of treatment, overall microbial activity figures were lower after the experiment than their respective starting values. The “combined” treatment significantly increased the generative growth of tomatoes. The number of flowers was significantly higher, and thus significantly more tomato was harvested when compared to the “control” treatment. Our preliminary results indicate the beneficial effects of *P. pruinosus*, because its presence had an advantageous influence on tomato yield.

Keywords: Oniscidea, soil fertility, woodlice, soil cover

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Introduction

Higher habitat and ecological diversity in a home-scale horticulture decreases the pressure from pests (Philpott 2013). Tomato production has an increasing trend both in the total area of production and the amount of harvest worldwide (FAOSTAT data, 2018). Tomato is typical to most home vegetables patches as well, with most of the gardeners cultivating more than one variety.

Consumers believe that landraces are nutritionally more valuable (Casals et al. 2011). While this argument has not yet been proven in all cases, genetic variability differs among various landraces and depends on selection pressure (Passam et al. 2007).

For our study we selected a Hungarian landrace (Dány) with the preconception in mind that landraces are better adopted to local conditions. We wanted to find a method that has the potential to increase the success of home-scale tomato production, to increase the amount of yield and the vitality of soil at the same time. In our study we were looking for providing production conditions that are relatively less experienced with yet are low on the budget and energy demand.

The benefits of using natural mulches are manifold. Suppressing weeds, preserving soil moisture and therefore, stabilizing soil structure are just some of them. One has to mention that besides contributing to the production of healthy, marketable crops, good mulching practice provides suitable conditions for beneficial organisms, making the use of mulch a factor of key importance within the complexity of crop protection (Mancinelli et al. 2015, Diver et al. 1999). Mancinelli et al. (2015) found that organic mulching, irrigation and fertilization decreased CO_2-emission and increased C-storage of the soil. There are many soil-dwelling living organisms involved in nutrient cycles to maintain soil fertility. Terrestrial isopods hide under dead plant matter, using it as shelter and food source at the same time (Stachursky 1968). Woodlice are also known for manipulating the soil: to consume decaying organic matter and release it back as faeces to the soil again. Within their intestinal tract, decaying materials are broken down to small, organic molecules, which are now available for the soil bacteria and fungi for the processes of immobilization, mineralisation and humification. This is how isopods have an indirect influence on the microbial activity of

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the soil (Zimmer and Topp 1999; Lavelle et al. 2006; Vilisics et al. 2012). *Porcellionides pruinosus* was found the most abundant species in compost heaps (Farkas and Vilisics 2013).

The positive effect of mulching has been well documented in recent times (Campiglia et al., 2011, Shirgure, 2012, Kumar, 2014), and the beneficial effects of faecal pellets of woodlice and millipedes on the growth of test plants were proven in pot experiments (Gere 1956; Farkas et al. 2017).

Therefore we expected mulching to increase the microbial activity of the soil, and tomato yield; and by adding (not faecal pellets but living individuals of) woodlice to the system, to further increase the beneficial effects of mulch. We also predicted that the lack of mulch would make woodlice leave the pots, and that in those pots there would be no significant change in either the microbial activity of the soil or in the yield.

Methods

Selection of plants and test animals

For our purposes, the regional cultivar Dány was used. This determinate, mid to late season variety is mainly for fresh consumption. It has a moderate growth vigour, and a strong foliage rejuvenation (ÖMKi 2015). The growing period for this variety was observed to start late March to early April with sowing, and to end mid to late September with the final harvests. Tomato seeds were sown in Florimo® general potting soil (*Sphagnum* moss peat, flat peat, organic humus, composted cattle manure, clay, fertilizer mixture, with a pH of 6.4±0.5). The plants were grown in greenhouse until the age of six weeks, when the seedlings were transplanted one by one into a pot. There were 40 pots with the same soil, one seedling per pot. Thereafter Isopods were placed into the pots with or without leaf litter.

Our test animal was Porcellionides pruinosus. Besides its simple availability and synanthropic lifestyle, this isopod became our test species because it responds well to various environmental circumstances and is easy to rear among artificial conditions.

Porcellionides pruinosus individuals were collected from the Regional Waste Management Center of Pusztazámor by handsorting. The animals were bred and kept at the Institute of Plant Protection of Szent István University. For identification we used the taxonomic key of Farkas and Vilisics (2013).

Experimental design

The study started on 24 May 2016 in the experimental area of the Plant Protection Institute of the Szent István University in Hungary (SZIE NVI) in a partially covered glasshouse and it was terminated on 23 September 2016, after the growing period. Before distributing the same amount of potting soil („Original soil") into every pot, the enzymatic activity of the soil was measured with *fluorescein diacetate*

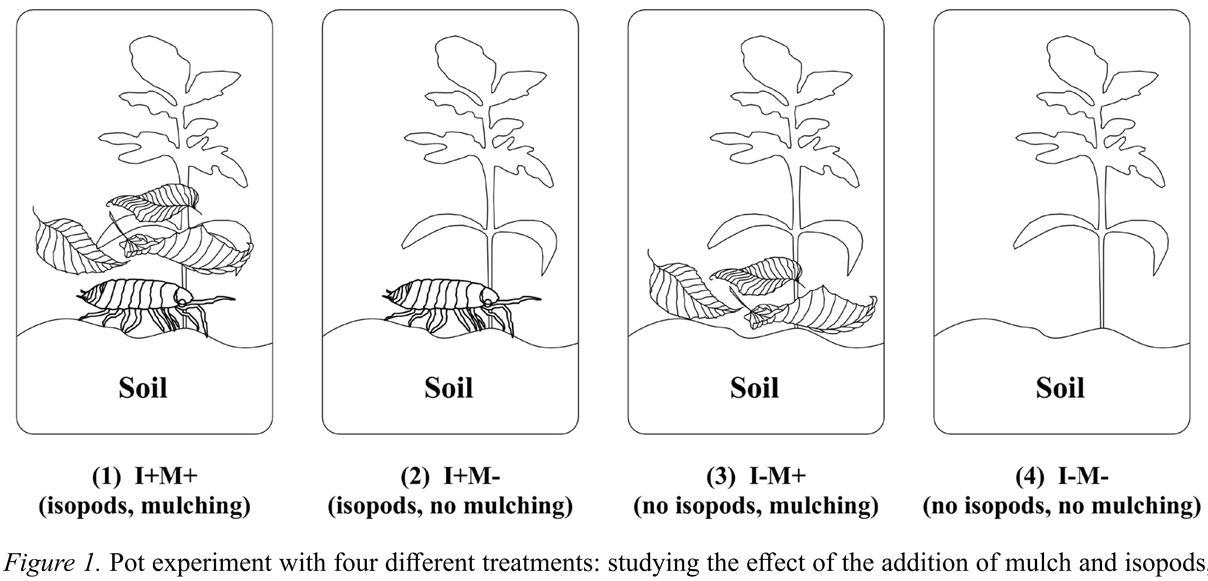

Figure 1. Pot experiment with four different treatments: studying the effect of the addition of mulch and isopods, using a tomato landrace variety as test plant

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(*FDA*) *hydrolysis assays* te ts. There were four treatments in 10 replicates as follows: (1) I+M+ (isopods, mulching); (2) I+M- (isopods, no mulching); (3) I-M+ (no isopods, mulching) and (4) I-M- (no isopods, no mulching) (Fig 1.).

Placement of Porcellionides pruinosus individuals and mulch

In each I+ pot, 20 specimen of *P. pruinosus* adults (with an average body weight of 228.5 mg) were placed. We chose to not differentiate between genders as this type of selection would have hurt the individuals, but gravid females were excluded. The number of individuals, adults and juveniles were counted at the completion of the experiment. Mulch material was collected from the surrounding areas: the mulch mixture contained walnut (*Juglans regia*), elm (*Ulmus minor*) and maple (*Acer platanoides*) leaf litter of local trees in a ratio of 1:1:1. Every mulched pot was covered with an average of 39.6 g of leaf matter. The soil of the pots was sieved at the end of the experiment, and the number of adult and juvenile isopods was recorded.

Soil microbial activity

To measure and monitor the microbial activity of soil, we followed the method of Schnürer and Rosswall (1982). Each pot was sampled before the experiment and at the end of the experiment again by taking 2 g of soil. Samples were incubated for two hours at 24°C with 10 ml sodium-phosphate buffer (60mM, pH= 7.6) and 100 µl of FDA (2 mg/l). The rotary shaker was set at 300 RPM. After two hours the reaction was halted by the addition of acetone (10 ml). The solution was sieved, and the amount of hydrolysed FDA was measured. Absorbance values were obtained by using a Haca DR/2000 spectrophotometer at 490 nm. Calibration curves were drawn using solutions of known fluorescein concentration, and thus, the amount of fluorescein obtained from our samples during the two-hour period was calculated. Each test was replicated ten times. The values were calculated for one gram of dry soil as mg of fluorescein.

Parameters of tomato

Both the tomatoes and their respective pots were sampled every fortnight during the growing season. The number of flower buds, flowers and leaves were recorded. Indicators of maturity: the number of fruits, their weight were also measured. The number of flower buds, flowers and fruits, and the total weight fruits per plant were regularly monitored. Root weight was measured at the end of the growing season.

Statistical analyses

Levene's test for homogeneity of variance, ANOVA with Tukey's posthoc test, Kruskal-Wallis test with Mann-Whitney pairwise comparisons and Welch test (PAST® program: Hammer et al 2001) were used for statistical analyses.

Results

Soil microbial activity

The fluorescein concentration values (µg/ml) of treatments revealed that the addition of mulch material resulted in higher microbial activity (Fig. 2) ($F=14.35$, $df=21.77$, $p=7.015 \times 10^{-6}$).

Regardless of the presence of isopods, there was a significant difference between treatments with or without mulch, that is, between treatmentsww

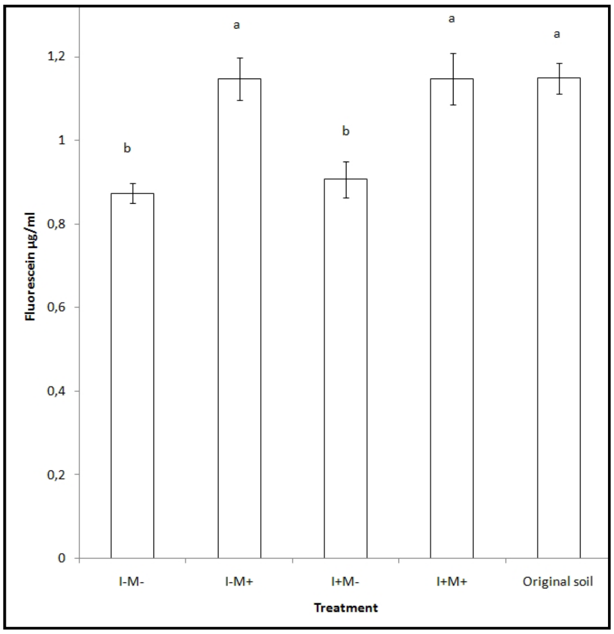

Figure 2. Total soil microbial activity by fluorescein concentrations (µg/ml) in a tomato pot experiment with mulch and the isopod *Porcellionides pruinosus* [I=Isopod, M=Mulch; the same letters above bars indicate the lack of significant ($p<0.05$) difference; error bar: ±SE; ANOVA, Tukey's posthoc test]

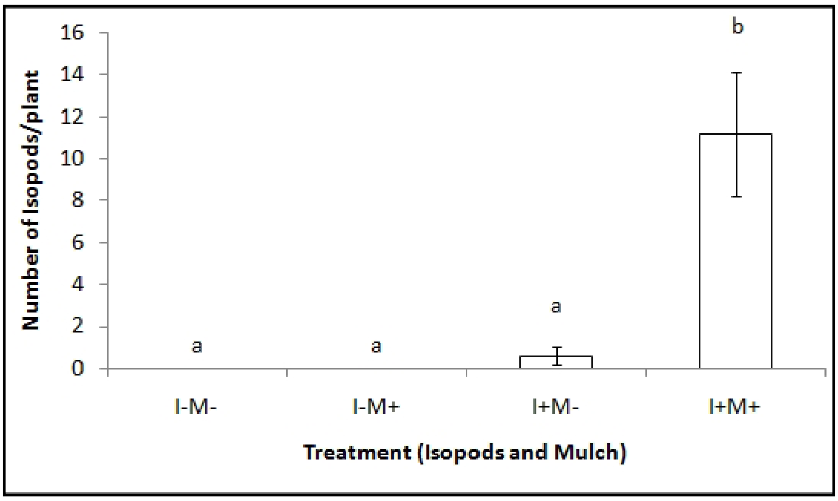

Figure 3. The number of *Porcellionides pruinosus* individuals at the end of a tomato pot experiment where isopods and mulch were both added to the soil [I=Isopod, M=Mulch; the same letters above bars indicate the lack of significant ($p<0.05$) difference; error bar: ±SE; Kruskal-Wallis test, Mann-Whitney pairwise comparisons]

I-M- and I-M+; and between treatments I+M- and I+M+. The fluorescein values measured for the two no-mulch treatments (I-M- and I+M-) were also significantly lower than the initial values (Original soil) measured prior to the pot experiment. Statistical analysis confirmed that the original microbial activity of the soil was not significantly different from the values obtained in treatments with mulch (I-M+ and I+M+) but was significantly higher than those without mulch (I-M-) and (I+M-) (Annex 1).

Porcellionides pruinosus and mulch

The number of *Porcellionides pruinosus* individuals at the end of the experiment was lower than initially, but mulching had a beneficial effect on number of *P. pruinosus* individuals (Fig.3., Annex 2) ($H=17.42$; $p=3.40\times10^{-6}$). The number of juveniles were also recorded, but due to low recapture no reliable conclusions were drawn from these data.

Parameters of tomato

Number of flower buds, flowers and leaves

No significant difference was found in the maximum number of flower buds and leaves between the treatments (Annexes 3-4) There was a significant difference however, in the maximal number of flowers ($F=5.246$, $df=19.83$, $p=0.008$; CI95% and average I-M- 0.682 and 3.9, I-M+ 0.787 and 5.5, I+M- 0.889 and 4.5,

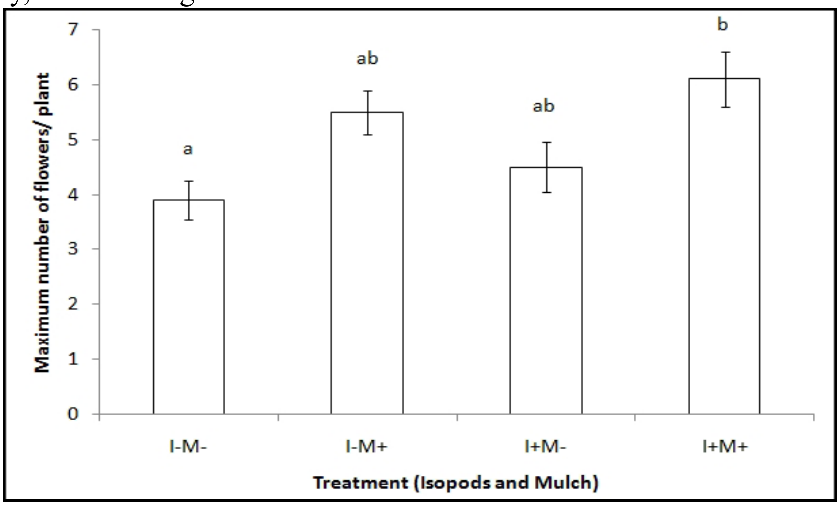

Figure 4. The maximum number of tomato flowers in a pot experiment studying the effect of *Porcellionides pruinosus* and mulch [I=Isopod, M=Mulch; the same letters above bars indicate the lack of significant ($p<0.05$) difference; error bar: ±SE; ANOVA, Tukey's post hoc test]

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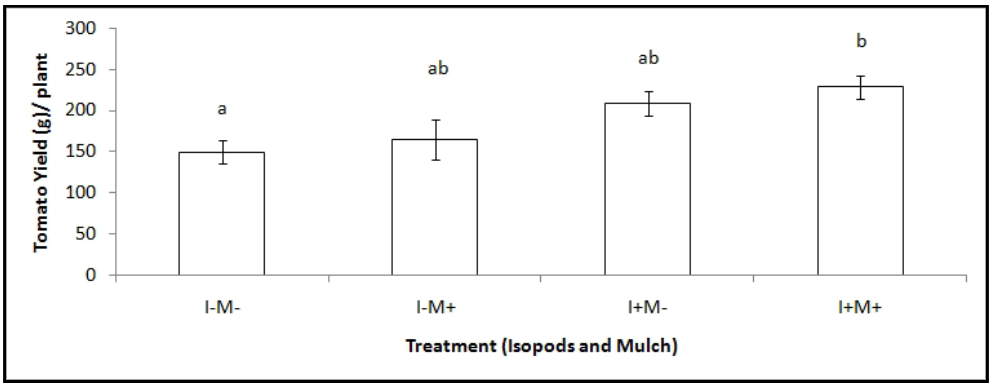

Figure 5. Tomato yield according to the presence or lack of a woodlice species, *Porcellionides pruinosus* and mulch in a tomato pot experiment [I=Isopod, M=Mulch; the same letters above bars indicate the lack of significant ($p<0.05$) difference; error bar: ±SE; ANOVA, Tukey's post hoc test]

I+M+ 0.989 and 6.1; Tukey's pairwise I+M+ 0.005) (Fig. 4, Annex 5).

When only isopods were added (I+M-), the decreasing trend in the number of flower buds and flowers was slower than in any of the other treatments.

The number of fruits, their weight and root weight

None of the treatments had a significant effect on the number of fruits per plant (p=0.079).

Neither the addition of isopods, nor the addition of mulch alone had a significant effect on fruit weight, but isopods and mulch together had a recognizable effect on tomato yield (F=5.78. df=19.76. p=0.005) (Annex 6).

The effect of adding isopods to pots on tomato yield is recognizable (p=0.013).

On the other hand, no significant difference was recorded when only mulch was added (M+). According to these results, the difference between the yield of tomato plants treated and those not treated with isopods was statistically not proven. None of the treatments alone had a significant effect on the number of fruits harvested, but there was a significant dissimilarity between the effect of the "combined" (I+M+) and the "control" (I-M-) treatment on the weight of the yield (Fig. 5).

Measuring the dry and fresh matter content of the roots revealed that none of the treatments had a significant influence on the growth of the root system (p = 0.486). Yield (fruit weight per plant) kept increasing continuously as the season wore on. The increase was the highest with the "mulch only" (I-M+) treatment; and its values were followed by the values of the "combined" treatment (I+M+) (Fig. 6).

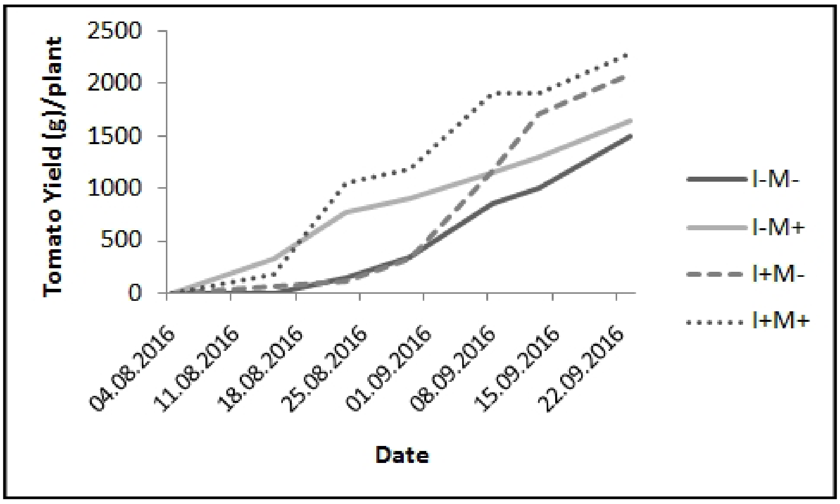

Figure 6. Cumulative tomato yield growth during the experiment with four treatments (I=Isopod, M=Mulch) in a tomato pot experiment

Discussion

Adding mulch alone did not have a significant effect on tomato yield, but a combination of mulch and isopods did. While Tuf and Tufová (2005) claimed that soil macroinvertebrates, due to their role in the ecosystem, increase soil fertility, we only recorded a significant effect of macroinvertebrates when combined with mulch.
Since the type and origin of mulch materials may have an impact on production indicators (Zribi et al. 2015), based on our previous studies (Fehér et al, 2017), we now decided to use organic mulch and in particular, the locally available leaf litter: an even mixture of walnut (*Juglans regia*), elm (*Ulmus minor*) and maple (*Acer platanoides*). Similarly to our experiment, Förster et al. (2006) also mulched with a various mixture of forest leaves, but in their set up faecal pellets of millipedes and woodlice were also added to crops, while we preferred to use the organisms themselves, which were found useful by Tantachasatid et al. (2017) in their experiment. Our prediction, based on the results of Förster et al. (2006) was proven: soil microbial activity declined in the absence of mulch in our treatments. Without a proper organic cover, soil was more prone to desiccation, its microbial activity decreased, and it also failed to provide isopods with habitat and shelter.

Within this limited timeframe of our present experiment mulch material did not have the time to enhance the nutrient content of the soil. We believe mulch provided a habitat and a shelter for our isopods as was found by Diekötter et al (2010). Similarly to a natural environment, isopods were not confined: they had the ability to leave the pots, and we assume that they hardly ever found their way back to the same, or to any pot. Where there were none at the beginning, none was found at the termination of the experiment either. No new isopods settled to pots, and migration was low, probably because it was hindered by the mulch leaves. In the end the number of juveniles was measured but it is not a reliable data because the sex ratio was not determined before the experiment.

Individuals of *Porcellionides pruinosus* contributed to leaves being decomposed and buried into the soil, although the contribution was definitely smaller than that of the dung beetle, also considered an ecosystem engineer (Johnson et al. 2016). This raises the question: would the impact of isopods on soil and plant have been higher had the isopods been introduced in considerably larger numbers. If so, is there an optimum number of individuals to be introduced. Is this optimum amount different for each isopod species, for soil types, and in general, what influence ecological conditions have on this data? To answer these questions, further studies are needed.

Conclusions

Our preliminary results indicate the beneficial effects of the combined application of leaf litter mulch and *Porcellionides pruinosus* on tomato yield.

The observation that isopods were found only in pots where they were introduced at the beginning of the experiment may suggest that these animals do not leave their micro-environment when the conditions were favourable for their survival. One may thus suspect that the useful influence of isopods is only present when the conditions favoured by these arthropods are created within the course of crop production protocol, that is, when mulching is part of the management.

We propose to test this innovative system in larger scale experiments. The biomass quantity of woodlice in our experiment was not remarkable enough and more experiments with higher isopod density are suggested.

We consider treating production areas with mulch and isopods a preliminary step in elaborating a new element of technology in crop production. We contemplate the possibilities of expanding our studies to a larger scale, to arable lands. At present, *P. pruinosus* is already a marketable item: it is reared mainly as prey (e.g. http://www.ebay.co.uk/itm/40-Isopods-*Porcellionides-pruinosus*-Dart-frog-Newts-and-Salamander-food-culture-/171520772125, https://www.insecte.org/forum/viewtopic.php?t=134763, https://argiope.se/ovogram/**)**. We suggest a series of detailed studies to take other steps towards the commercial sale of isopods to enhance soil fertility.

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Annexes

Annex1: **Soil microbial activity**

	I-M-	**I-M+**	**I+M-**	**I+M+**	**Original soil**
Average	0.8736	1.1477	0.9068	1.1473	1.1486
Deviation	0.075020294	0.1602	0.13805297	0.1943	0.115313293
SE	0.0237235	0.0506	0.04365618	0.0614	0.036465265
CI 95%	0.046497205	0.0993	0.08556454	0.1204	0.071470606

One-way ANOVA

	Sum of sqrs	df	Mean square	F	p(same)
Between groups:	0.802225	4	0.200556	9.891	7.892E-06
Within groups:	0.912425	45	0.0202761		
Total:	1.71465	49			
omega^2:	0.4156				

Levene's test for homogeneity of variance. based on means: p(same) =	0.7645
Based on medians: p(same) =	0.8287
Welch F test in the case of unequal variances:	F=14.35. df=21.77. p=7.015E-06

Tukey's pairwise comparisons: Q below diagonal. p(same) above diagonal

	I-M-	I-M+	I+M-	I+M+	Original soil
I-M-		0.0009404	0.9848	0.0009562	0.0009059
I-M+	6.087		0.004092	1	1
I+M-	0.7373	5.35		0.004165	0.003932
I+M+	6.078	0.008883	5.341		1
Original soil	6.107	0.01999	5.37	0.02887	

Annex2: ***Porcellionides pruinosus* and mulch**

Kruskal-Wallis test for equal medians

H (chi2):	17.42
Hc (tie corrected):	28.14
p (same):	3.40E-06

There is a significant difference between sample medians

Mann-Whitney pairwise comparisons. raw p-values. uncorrected significance

	I-M-	I-M+	I+M-	I+M+
I-M-		1	0.1681	0.0002312
I-M+	1		0.1681	0.0002312
I+M-	0.1681	0.1681		0.001151
I+M+	0.0002312	0.0002312	0.001151	

Annex3: Maximum number of flower buds

	Average	CI 95%				
I-M-	7.2	1.998778921				
I-M+	6.3	2.08756356				
I+M-	7.7	1.802267141				
I+M+	8.6	1.466702744				
One-factor variance analysis						
Summary						
Groups	*Quantity*	*Sum*	*Average*	*Variance*		
I-M-	10	72	7.2	10.4		
I-M+	10	63	6.3	11.34444444		
I+M-	10	77	7.7	8.455555556		
I+M+	10	86	8.6	5.6		
Variance analysis						
Factors	*SS*	*df*	*MS*	*F*	*p-value*	*F crit.*
Between groups	27.7	3	9.233333333	1.031657356	0.3901503	2.866266
In groups	322.2	36	8.95			
Sum	349.9	39				

Annex4: **Maximal number of leaves**

	Average	CI 95%				
I-M-	12.1	1.110646258				
I-M+	11.8	0.703648605				
I+M-	12.5	0.602332503				
I+M+	12.8	1.124016514				
One-factor variance analysis						
Summary						
Groups	*Quantity*	*Sum*	*Average*	*Variance*		
I-M-	10	121	12.1	3.211111111		
I-M+	10	118	11.8	1.288888889		
I+M-	10	125	12.5	0.944444444		
I+M+	10	128	12.8	3.288888889		
Variance analysis						
Factors	*SS*	*df*	*MS*	*F*	*p-value*	*F crit.*
Between groups	5.8	3	1.933333333	0.885496183	0.4578358	2.866266
In groups	78.6	36	2.183333333			
Sum	84.4	39				
One-way ANOVA						
	Sum of sqrs	df	Mean square	F	p(same)	
Between groups:	5.8	3	1.93333	0.8855	0.4578	
Within groups:	78.6	36	2.18333			
Total:	84.4	39				
omega^2:	-0.008662					
Levene's test for homogeneity of variance. based on means: p(same) =	0.355					
Based on medians: p(same) =	0.5658					
Welch F test in the case of unequal variances:	F=1.004. df=19.41. p=0.4124					
Tukey's pairwise comparisons: Q below diagonal. p(same) above diagonal						
	I-M-	I-M+	I+M-	I+M+		
I-M-		0.9685	0.9298	0.7161		
I-M+	0.642		0.7161	0.4404		
I+M-	0.8561	1.498		0.9685		
I+M+	1.498	2.14	0.642			

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Annex5: **Maximum number of flowers**

	I-M-	I-M+	I+M-	I+M+	
Average	3.9	5.5	4.5	6.1	
Deviation	1.100504935	1.269295518	1.433720878	1.595131482	
SE	0.348010217	0.401386486	0.45338235	0.504424865	
CI 95%	0.682087492	0.786703056	0.888613078	0.988654568	
One-way ANOVA					
	Sum of sqrs	df	Mean square	F	p(same)
Between groups:	29.2	3	9.73333	5.246	0.004167
Within groups:	66.8	36	1.85556		
Total:	96	39			
omega^2:	0.2415				
Levene's test for homogeneity of variance. based on means: p(same) =	0.6626				
Based on medians: p(same) =	0.7355				
Welch F test in the case of unequal variances:	F=5.246. df=19.83. p=0.007889				
Tukey's pairwise comparisons: Q below diagonal. p(same) above diagonal					
	I-M-	I-M+	I+M-	I+M+	
I-M-		0.0583	0.7589	0.004985	
I-M+	3.714		0.3691	0.7589	
I+M-	1.393	2.321		0.0583	
I+M+	5.107	1.393	3.714		

Annex6: **Tomato Yield**

	I-M-	**I-M+**	**I+M-**	**I+M+**	
Average	149.69	164.88	209.9	228.94	
Deviation	44.67739796	75.84869149	46.58826748	44.82772456	
SE	14.12823375	23.98546226	15.05652313	14.17577119	
CI 95%	27.69082931	47.01064219	29.51024308	27.78400099	
One-way ANOVA					
	Sum of sqrs	df	Mean square	F	p(same)
Between groups:	41573.9	3	13858	4.647	0.00759
Within groups:	107362	36	2982.27		
Total:	148936	39			
omega^2:	0.2148				
Levene's test for homogeneity of variance. based on means: p(same) =	0.006713				
Based on medians: p(same) =	0.009981				
Welch F test in the case of unequal variances:	F=5.78. df=19.76. p=0.005236				
Tukey's pairwise comparisons: Q below diago-nal. p(same) above diagonal					
	I-M-	I-M+	I+M-	I+M+	
I-M-		0.9244	0.08299	0.01304	
I-M+	0.8796		0.2704	0.05875	
I+M-	3.487	2.607		0.8632	
I+M+	4.589	3.709	1.103		