SEASONAL CHANGES OF SOME MICROBIOLOGICAL PROPERTIES OF SOILS IN A FIELD OF HAZELNUT (CORYLUS AVELLANA L.) GROWING

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Abstract. The aim of this study is to identify the effects of the compost obtained from hazelnut husks by using biological techniques, on soil quality at different sampling times. The composted hazelnut husk was used at 6 application doses and with 3 replications in order to increase the organic material content of the soils at doses of 0, 0.5%, 1%, 2%, 3% and 4%. The hazelnut husk compost was applied at the Akçatepe/Ordu/Turkey experiment area on the 23rd of November 2012 by using the mattock planting method. The soil samples were collected from the field at 4 different sampling seasons and consequently, various chemical and biological soil characteristics were detected. The results shows; certain doses and different sampling seasons have a significant effect on the beta-glucosidase, urease and acid phosphatase enzyme activities in soils; the most effective doses were 4% and 3%; the 1st and 4th seasons provided the best results. As for other biological characteristics the highest values were as follows: CO₂ production at 1% dose, microbial biomass-C value at 2% dose. The application of hazelnut husk compost, which has a rich organic matter content and is an organic material itself, to soils, promotes an increase in soil organic materials.

Keywords: hazelnut husk, compost, soil enzymes, organic material, microbial biomass-C

Introduction

The use of wastes, which have an important place in agricultural production, as an organic matter source for soils, has recently become a common practice. The utilization of wastes is also a solution for environmental pollution. Especially following the hazelnut (Corylus avellana L.) harvest season, its husk is either used as fuel, as cushions for animals or left aside as idle wastes. Especially in Turkey’s Black Sea region, hazelnut is an important product. It is known that approximately 550,000 tons of shelled hazelnut is produced annually in this region. After the harvest, from 1 kg of fresh hazelnut, remains around 1/3 and from shelled hazelnut 1/5 (200 gr) of dry husk shell (Çalışkan et al., 1996; Bender Özenç, 2008; Amiri et al., 2017).

Turkey is the world’s leading hazelnut producer, accounting for about 75% of world hazelnut supply. According to FAO data, Turkey is the first world hazelnut producer and exporter that covers approximately 70% and 82% of the world’s production and export respectively. It is followed by Italy with nearly 20% in production and 15% in export. That is why the Turkey is at the focus of the international academic studies about hazelnut, hazelnut husk compost etc. and references about these issues (FAO, 2019). Solely 10% of the hazelnut consumption is accounted in-shell, the rest of this ratio is shelled and evaluated for industrial objectives (Tous, 2001; Stévigny et al., 2007).

Especially in Giresun, Ordu, Trabzon and Samsun in the Black Sea Region and Sakarya in the Marmara Region, hazelnut cultivation is the main source of living. The Black Sea Region’s ecological structure is suitable for hazelnut production. Thus
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Hazelnut is an essential product in Turkey's economy. Most densely in the Black Sea Region, hazelnut is produced in 39 provinces of Turkey. Hazelnut production is mainly run as family-owned businesses. According to Republic of Turkey Ministry of Agriculture and Forestry, around 395 thousand families are economically focused on hazelnut production with a total area of 700 thousand hectares (GTB, 2013). The increase in world's population, accelerates the agricultural activities on soils. The excessive and unconscious use of fertilizers and chemicals common in traditional agricultural system, generates both environmental and soil issues. Most of soil issues arise from the wrong or misuse of it, which ends up with the soil impairment. The soil is a natural and living substance, which together with air and water contains millions of microorganisms and provides an environment for human beings, plants and animals to live. Even the smallest piece of land needs thousands of years to form; hence, our soils are invaluable within the ecosystem. Especially the benefits provided by the environment that soils render for plant production, show how crucial it is for human being's life. A healthy soil means a healthy plant production environment, which eventually connotes that plants grown on such lands will be healthier. In order to ensure healthier plants, to protect their quality features, fertility and sustainability, it is necessary to protect the soil idiosyncrasy. All these features are related to the quality of soils, the sustainability or developability of some characteristics, which appear in the soil quality (Aygün, 2015).

Microbial biomass carbon, CO₂ production (soil respiration) and dehydrogenase activity are commonly used parameters to identify the biological effects of organic and inorganic matters penetrated into the soils (Meli et al., 2002).

The application of hazelnut husk compost into the soils, provides an increase by means of organic matter, ensuring as a consequence many of its physical, chemical and biological features to improve. This application radically improved the microbial activities in the soil, by means of soil buffer capacity (thus chemical soil properties like pH, electrical conductivity, the amount of exchangeable cations, organic matter and total amount of nitrogen content; by means of physical properties: aggregate stability, bulk density, field capacity and wilting point; by means of biological properties: soil respiration and biomass-C content increased (Birol and Bender Özenç, 2011; Aygün, 2015; İslam, 2016).

The application of clinoptilolite, leonardite, organic commercial fertilizer (biofarm) and two different types of hazelnut husk (fresh and composted), which is a regional waste, as soil regulators in a certified organic hazelnut garden revealed positive postharvest developments by means of soil enzymes (dehydrogenase, urease, phosphatase and sulfatase) and the amount of microbial biomass (Özyazıcı et al., 2011).

Soil enzymes are basically produced by soil microorganisms, which are able to show the microbial activities observed in the soil environment and play a crucial role during the decomposition of organic matters and transformation of plant available nutrition elements (Xiu-Mei et al., 2008).

The aim of this study; is to examine the relationship between the effects of the application of the compost, at varying doses, obtained from hazelnut husk by using biotechnological techniques and the effects of sampling seasons on various biological and chemical properties of the soil.
Materials and methods

The experiment was carried out in the high tunnel greenhouse established in Ordu University experiment area. The study area is located in Ordu province, Altınordu district, Eastern Black Sea Region, Turkey in 2016. The province of Ordu is located between the northern latitudes of 37° 45' 34''765, with eastern longitudes of 41° 16' 38'' (Fig. 1).

Figure 1. Map shows the location of Akçatepe district experiment area in the Ordu Province, Turkey

In this study, the compost of hazelnut husk, which was obtained by using microbial biotechnological methods, has been used as material. The field test (Akçatepe District) was conducted on the 23rd of November 2012; varying application doses of hazelnut husk compost were applied in order to increase the soils organic material content as of 0 (control), 0.5% (0.125 kg ha⁻¹), 1% (0.25 kg ha⁻¹), 2% (0.50 kg ha⁻¹), 3% (0.75 kg ha⁻¹) and 4% (1 kg ha⁻¹); the compost was applied uniformly to a 50-60 cm diameter circle around the hazelnut plots by mattocking and mixing with soil to a 10-15 cm depth. The sampling was realized in four sampling seasons: autumn (I, 25 November 2016), winter (II, 23 February 2017), spring (III; 24 May 2017) and summer (IV, 22 August 2017). The tests were carried out according to randomized blocks trial design with three replications (Aygün, 2015). Hazelnut husk (C/N ratio 55.71; pH 5.81; EC25 °C
1.93 dS m\(^{-1}\); 0.97% N) was collected from the hazelnut orchard, inoculated with carbon and the microorganisms used as energy sources were composted by windrow method and eventually used as a material in experiments using a windrow machine in the Research Facility of Soil Science and Plant Nutrition Department at Ondokuz Mayıs University, Samsun, Turkey. HHC properties are as follows: pH is 6.76, EC25 °C is 3.56 dS m\(^{-1}\), organic matter (OM) content is 94.75%, total N content is 2.48% and C/N ratio is 22.16 (Kızılkaya et al., 2015a,b).

The loamy soil of the experiment land in Akçatepe shows a neutral ground reaction and a saline electrical conductivity (Table 1). The lime (CaCO\(_3\)), organic matter content, total nitrogen and available phosphorus contents in Akçatepe soil were adequate (Aygün, 2015).

Table 1. The soils physico-chemical properties (Aygün, 2015)

| Analyses                      | Akçatepe Dist. (Ordu Province) |
|-------------------------------|--------------------------------|
| % sand                        | 33.55                          |
| Texture % silt                | 27.86                          |
| % clay                        | 38.59                          |
| Textural class                | Loamy soil (CL)               |
| Soil reaction-pH (1/2.5)      | 6.69                           |
| Electrical conductivity (dS m\(^{-1}\)) (1/2.5) | 1.43                        |
| Lime content (CaCO\(_3\)), %  | 5.23                           |
| Organic matter, %            | 2.58                           |
| Total N, %                    | 0.196                          |
| Available P, mg kg\(^{-1}\)  | 15.39                          |
| Exchangeable Na, cmol(+) kg\(^{-1}\) | 0.326                       |
| Exchangeable K, cmol(+) kg\(^{-1}\) | 0.444                       |
| Exchangeable Ca, cmol(+) kg\(^{-1}\) | 39.90                        |
| Exchangeable Mg, cmol(+) kg\(^{-1}\) | 1.26                        |

Soil physico-chemical analyses

Soil samples, grated to 2 millimeters were kept waiting and prepared for the analyses in + 4 °C refrigerator immediately after, and biological analyses were initiated. To identify the properties of the sample soil, the texture was examined by hydrometer method as told by Bouyoucos (1962), soil reaction (pH) and saltiness (EC) by U.S. Salinity Laboratory Staff (1954), organic material by Nelson and Sommers (1982), total nitrogen by Bremer (1965), extractable potassium by Knudsen et al. (1982) as told by Müftüoğlu et al. (2012).

Soil biological analyses

For the biological analyses of the soil; CO\(_2\) production was identified using the method told by Isermeyer (1952); microbial biomas-C by Anderson and Domsch (1978), β-Glucosidase Enzime activity Eivazi and Tabatabai (1988), urease enzyme activity by Kandeler and Gerber (1988), acid phosphatase enzyme activity by Tabatabai and Bremer (1969) as told by Schinner et al. (1996).
**Statistical analysis**

At the end of the study, the variance analysis of the obtained data was performed using the JMP package program and the important results were evaluated according to the LSD test.

**Results and discussion**

The effects of the beta-glucosidase enzyme activity (p < 0.01), urease enzyme activity (p < 0.01) and acid phosphatase enzyme activity (p < 0.01) were relevant to application and season interactions effect revealed statistically significant results (Table 2). When the application doses of hazelnut husk compost and sampling seasons are taken into consideration, the highest β-glucosidase enzyme activity occurred at 0.1 kg ha\(^{-1}\) compost dose on the 4th season (summer), this value has been followed by the 2nd season (winter) application with a compost dose of 0.25 kg ha\(^{-1}\) and the lowest control was detected on the 1st season (autumn). As both the hazelnut husk compost application doses on the 4th season (summer) and sampling seasons multiplied, the beta-glucosidase enzyme activity of soils increased accordingly. It has been stated that the soil organic matter absorbs the beta-glucosidase enzyme and therefore induces the activity in the soil to continue (Wang et al., 2006). The β-Glucosidase enzyme activity is one of the essential soil enzymes, which is responsible for the C cycle (Nannipieri et al., 2002; Kayıkçıoğlu and Okur, 2011).

The urease enzyme activity’s peak (73.55) occurred on the 1st season (winter) at a dose of 0.75 kg ha\(^{-1}\), this was followed by the 0.125 kg ha\(^{-1}\) dose and these two doses were identified as the most effective ones. The lowest urease enzyme activity dose was 0.50 kg ha\(^{-1}\) and it was identified on the 4th season (summer). The applications of hazelnut husk increased the soils urease enzyme activity; however, during the summer season the increasing temperature accelerated the decomposition of organic materials, resulting in the 4th sampling season (summer) to reveal the lowest urease enzyme activity values.

**Table 2. The effects of application seasons of the hazelnut husk compost on the β-glucosidase, urease and acid phosphatase enzyme activities**

| Doses (kg ha\(^{-1}\)) | 1.P | 2.P | 3.P | 4.P | Doses (kg ha\(^{-1}\)) | 1.P | 2.P | 3.P | 4.P | Doses (kg ha\(^{-1}\)) | 1.P | 2.P | 3.P | 4.P |
|-----------------------|------|------|------|------|-----------------------|------|------|------|------|-----------------------|------|------|------|------|
| 0                     | 1.98g | 7.03efg | 8.91efg | 1.76g | 0 | 12.58e-g | 16.97e-g | 9.96e-g | 5.26f | 0 | 6.01c-f | 5.32c-f | 8.25c-f | 1.76d |
| 0.125                 | 2.15f | 13.42e-g | 12.13c-g | 8.28e-g | 0.125 | 67.93a | 22.74c-d | 18.61d-g | 5.66f | 0.125 | 6.61c-f | 4.70d-f | 11.23c-d | 8.28c-f |
| 0.25                  | 4.75efg | 28.83ab | 15.11c-f | 10.47d-g | 0.25 | 63.26ab | 29.70c-e | 13.44e-g | 5.45f | 0.25 | 12.34c | 7.45d-f | 30.34ab | 10.47cd |
| 0.5                   | 3.52fg | 15.1c-f | 17.81b-c | 4.14f | 0.5 | 43.40bc | 24.67c-d | 19.52d-g | 4.14f | 0.5 | 9.20c-e | 4.90d-f | 23.04b | 4.13d-f |
| 0.75                  | 2.78fg | 25.07abc | 22.35a-d | 2.5f | 0.75 | 73.55a | 20.95d-g | 22.15d-g | 5.25g | 0.75 | 8.40c-f | 6.09c-f | 27.16ab | 2.50ef |
| 1                     | 3.23fg | 10.61d-g | 13.28c-g | 32.15a | 1 | 30.20c-e | 37.87cd | 25.21c-f | 5.78f | 1 | 6.57c-f | 5.57c-f | 30.14ab | 32.15a |

As the strongest acid phosphatase enzyme activity occurs in the beta-glucosidase enzyme activity, a 1 kg ha\(^{-1}\) dose application of the hazelnut husk compost on the 4th sampling season (summer) revealed the highest values (32.15 µg P- N g.d.s\(^{-1}\)), whereas the lowest acid phosphatase enzyme activity value was identified during an application of a 1.25 kg ha\(^{-1}\) dose and on the 2nd (winter) sampling season (4.70 µg P- N g.d.s\(^{-1}\)). In

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previous studies, the effects of vegetable substances like wheat and corn stalk and as animal products, the effects of horse, hog and cow manure on soils were investigated, ultimately it was determined that all these applications boost the urease, phosphatase and interphase activities, revealing the lowest rate of increase in the interphase activity and the highest in urease and phosphatase activities (Guan, 1989; Aygün, 2015).

Among the application doses of the hazelnut husk compost, the highest soil respiration (0.60 mg CO₂/24 h/g) was detected at the 0.25 kg ha⁻¹ application dose and the lowest (0.42 mg CO₂/24 h/g) at the compost’s control application. The difference between these values was statistically highly significant (p < 0.01). Furthermore, through the application of this dose, the CO₂ production value revealed a 1.4 times increase comparing to the control application (Table 3).

Considering the sampling seasons, the highest CO₂ production value was identified on the 1st and 4th seasons (autumn and summer) (0.30 mg CO₂/24 h/g), whereas the lowest value was identified on the 3rd season (spring) (0.21 mg CO₂/24 h/g). The difference between these seasonal values was statistically highly significant (p < 0.01). The CO₂ formation in agricultural soils usually varies between 3.5 and 35 mg CO₂-C 100 g.d.s⁻¹ values (Alexander, 1961; Kayıkçıoğlu and Okur, 2013). It has been stated that the CO₂ amount in organic farms is higher than in conventional agriculture farms (Melero, 2006; Tuomisto et al., 2012).

### Table 3. The effects of application doses of the hazelnut husk compost on the sampling seasons, CO₂ production (mg CO₂/100 g day⁻¹) based on doses, microbial biomass C (MBC mg biyomas-C 100 g.d.s⁻¹) and other soil properties (organic matter OM: %, total N %, K: mg kg⁻¹, Mg and Ca: cmol(+)/kg⁻¹)

| Doses  | Season | CO₂   | MBC   | OM    | Total N | K     | Mg    | Ca    | pH    |
|--------|--------|-------|-------|-------|---------|-------|-------|-------|-------|
|        |        | 0     | 1.25  | 2.5   | 5       | 7.5   | 10    | 1     | 2     | 3     | 4     |
|        |        | 0.42b | 0.58a | 0.60a | 0.58a   | 0.54a | 0.52a | 1.30a | 0.28c | 0.21c | 1.30a |
|        |        | 42.60b| 68.83a| 67.50a| 74.85a  | 70.13a| 69.16a| 24.82c| 52.05b| 14.20c| 170.98a|
|        |        | 1.60  | 1.71  | 1.65  | 1.41    | 1.38  | 1.61  | 1.43b | 1.93a | 1.35b | 1.53ab|
|        |        | 0.13  | 0.14  | 0.13  | 0.12    | 0.13  | 0.13  | 0.10c | 0.50a | 0.13bc| 0.14ab|
|        |        | 162b  | 176b  | 185b  | 199b    | 202b  | 266a  | 204   | 207   | 205   | 178   |
|        |        | 5.09c | 6.12bc| 6.72ab| 5.97bc  | 7.70a | 6.90a | 8.40a | 5.60b | 6.04b | 5.60b |
|        |        | 26.82a| 24.41ab| 24.28abc| 22.17bc| 20.71cd| 17.36d| 18.22c| 26.47a| 23.84ab| 22.00b|
|        |        | 6.70a | 6.36ab| 6.23b | 6.20b   | 6.10b | 6.03b | 6.52a | 6.16b | 6.32ab| 6.08b |

When the application doses of hazelnut husk are taken into consideration, the microbial biomass C (MBC) value was between 42.60 - 74.85 mg biomass-C 100 g.d.s⁻¹ and the difference between the hazelnut husk doses were statistically insignificant (p < 0.01). The doses have increased the MBC values comparing to the control and the highest value was identified at a dose of 0.5 kg ha⁻¹.

When the sampling season is considered, the MBC value showed a regular increase from the 1st (autumn) season to the 4th (summer) season (except the 3rd- spring season); the highest value arose on the 4th season (summer) and the difference between seasons was statistically significant (p < 0.01).
In respect to hazelnut husk doses, organic matter (OM) content increased and this increment showed seasonal changes. The effects of doses were statistically insignificant, however the highest value has been observed at a dose of 0.125 kg ha\(^{-1}\). When taking the sampling seasons into account, the highest organic material value was detected on the 2nd season (winter). The loamy soils own high surface areas and a high cation exchange capacity, thus showing protective features against the fragmentation of organic substances (Giller et al., 1997; Aygün, 2015). It was reported that organic waste applications radically improve the chemical features of loamy sand soils like the content of organic matter and exchangeable cations and enhance the soil quality (Candemir and Gülser, 2011). There are also other studies, which explain that hazelnut husk may be utilized as an organic material and nutritional source, which enhances the physical properties of the soils (Zeytin and Baran, 2003; Bender Özenç et al., 2019).

Garcia et al. (2004) investigated the effects of various plants on pH, EC, total organic carbon and carbon fractions, microbial biomass carbon, soil respiration, dehydrogenase, phosphatase, β-glycosidase and urease characteristics for 6 years. At the end of the researches they reported that; the aggregate stability, soil respiration, dehydrogenase, urease and phosphatase activities occur more strongly in plant growing soils; pH (7.5-8.0) and EC levels are lower compared to control; the total organic carbon and microbial biomass carbon available in the rhizosphere zone are higher compared to control application.

In consequence of statistical evaluations, the effects of hazelnut husk applications on total N were found insignificant, remaining within an interval of % 0.12-0.14 and showing the highest results at a dose of 0.25 kg ha\(^{-1}\). However the sampling season was statistically significant (\(p < 0.01\)). When sampling seasons are considered, it was determined that the 2nd season (winter) revealed the highest total N content (0.50%), while the 1st season (autumn) demonstrated the lowest total N content (0.10%). Shenbagavalli and Mahimairaja (2012) on the other hand reported that as the incubation season extends, the mineral N values radically reduce. Besides, as an expected result, when the microorganisms decompose the organic materials in the soil in order to create a source of nitrogen, the nitrogen content of the soil decreases (Bender Özenç et al., 2019).

The effects of hazelnut husk compost doses on extractable K were significant (\(p < 0.01\)). The application of the hazelnut husk compost in different doses, increased the extractable K content of the soil. The highest K value was detected at a dose of 1 kg ha\(^{-1}\) (266 mg kg\(^{-1}\)) and increased 1.65 times compared to control.

In terms of the exchangeable Mg and Ca values, doses and sampling seasons were statistically significant (\(p < 0.01\)). According to doses, the exchangeable Mg value was between 7.70-5.09 cmol\(_{(+)}\)kg\(^{-1}\) and the highest dose was 0.75 kg ha\(^{-1}\), the exchangeable Ca value on the other hand was between 17.36-26.82 cmol\(_{(+)}\)kg\(^{-1}\), showing the highest value at the control dose. The exchangeable Mg and Ca values were statistically significant in consideration of sampling seasons. The exchangeable Mg highest value (8.40 cmol\(_{(+)}\)kg\(^{-1}\)) was identified on the 1st sampling season (autumn), while it was the lowest (5.6 cmol\(_{(+)}\)kg\(^{-1}\)) during the 2nd (winter) and 4th (summer) sampling seasons. The exchangeable Ca value was between 18.22-26.47 cmol\(_{(+)}\)kg\(^{-1}\) (2nd-winter and 1st-autumn season).

When the soil reaction is analyzed, the effects of the hazelnut husk compost on doses and sampling times revealed statistically significant results (\(p < 0.01\)). The highest pH value was identified at the control application (6.70), the lowest pH value on the other
hand was determined at a compost application dose of 1 kg ha$^{-1}$ (6.03). And when the sampling seasons are examined, the highest value was observed at the 1st (autumn) (6.52) and the lowest at the 4th (summer) season (6.08).

**Conclusion**

Within the scope of this study, where the effects of different application doses and sampling seasons of hazelnut husk compost, obtained through microbial biotechnological methods, on the soil various biological features were examined, the biological soil properties showed improvements and positive developments. While the beta-glucosidase and acid phosphatase enzyme activities were the highest at a dose of 1 kg ha$^{-1}$, the most effective sampling season was the 4th (summer) one. Urease enzyme activity on the other hand was more effective during the 1st (autumn) season and the 0.75 kg ha$^{-1}$ dose was sufficient. For the production of CO$_2$ the 0.25 kg ha$^{-1}$ dose, for MBC the 0.5 kg ha$^{-1}$ dose, for total N and organic matter content the 0.125 kg ha$^{-1}$ dose were sufficient. To blend the hazelnut husk compost, which is an organic material containing a large amount of organic matter, with the soil, provided the soil organic matter content to increase, which ultimately was an intended result. Increased doses of hazelnut husk compost to the soil, although there is an increase in the amount of organic matter due to dose increase. The optimum level of the compost to be applied must be decided, the initial organic matter content of the soil is recommended.

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