DOI: 10.34101/ACTAAGRAR/1/16404

Soil microbial biomass and community responses to long-term tillage and fertilizer regimes in corn under corn-winter wheat rotation

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SUMMARY

Agrotechnical interventions, namely, tillage and fertilization have a great influence on soil microbial activities and biomass, hence it is important to investigate their effect in long-term experiments. This study aimed at evaluating the impact of long-term tillage and NPK mineral fertilizer application on soil microbiological parameters in corn grown under corn-winter wheat rotation. The soil samples were collected in June of 2024 from the long-term experiment of the University of Debrecen at Látókép established in 1991. The treatments included control (no fertilizer), NPK fertilization (160 kg ha^{-1} N, 60 kg ha^{-1} P_2O_5, 90 kg ha^{-1} K_2O) and tillages (Moldboard tillage-MT, Strip tillage-ST and Ripper tillage-RT). The soil microbial biomass and composition of its community (Actinomycetes, saprophytic fungi, Gram-positive bacteria, Arbuscular mycorrhizal fungi, Gram-negative bacteria) was measured by PLFA analysis of soil extract. Results indicate that NPK fertilization affected only the biomass of Arbuscular mycorrhizal fungi and Actinomycetes, whereas tillage greatly influenced the soil microbial biomass and community composition for all the microbial groups. Generally, the microbial biomass and community composition were highest under RT followed by ST and lowest under MT. In conclusion, conservation tillages are more favorable for soil microbial life than conventional tillage.

Keywords: *tillage; NPK fertilization; microbial biomass; corn*

INTRODUCTION

Corn (*Zea mays* L.) is regarded as one of the key crops cultivated globally (Ni et al., 2024) and is ranked as the second most extensively cultivated crop annually at a global scale (Aghaei et al., 2022). Currently, corn is used for various purposes ranging from food for human consumption, animal feed, industrial feedstock for biofuels, chemical compounds, pseudoplastics, as well as other materials (García-Lara & Serna-Saldivar, 2019) and is ranked among the most important food crops at a global scale (Shiferaw et al., 2011). However, the productivity of corn is linked closely to soil health.

Soil health refers to the soil's ability to serve as a long-term vital biologically active ecosystem for humans, plants and animal sustenance, while also serving as a bridge between agricultural and soil science, policy, stakeholder needs and sustainable-supply chains (Lehmann et al., 2020); as capacity of soil to function within the confines of the ecosystem to support animal and crop performance, maintain or enhance environmental quality, and globally improve human well-being (Yang et al., 2020), and, as a state of balance within the ecosystem and the functionality of a soil and its ability to support a stable ecosystem with substantial below and above ground biodiversity and productivity (Cardoso et al., 2013). As such, healthy soil being living and dynamic offers a wide range of ecological services, for example atmospheric greenhouse gas removal, nutrient recycling regulation, as well as ensuring clean water supply and plant productivity (M. Tahat et al., 2020).

In order to understand the concept of soil health, a number of indicators of what a healthy soil entails have been developed with the objective of measuring and assessing the changes in soil properties and functioning so as to comprehend soil health as an instrument for sustainability (Raghavendra et al., 2020). These include biological, chemical and physical properties that promote ecosystem functioning and sustainable agriculture. Thus indicators of soil health are a combined set of quantifiable chemical, biological and physical characteristics which relate to functional soil attributes (Crittenden et al., 2024; Raghavendra et al., 2020). A number of factors key for agriculture productivity are involved in regulating soil health, among which include the availability of macro/micronutrients, indicator enzymes of soil health and microbial diversity as well as physicochemical soil properties (Chaudhary et al., 2022).

In agro-ecosystems, shifts in soil health can occur due to man-made activities such as cropping practices preferred by the farmer as well as intensive farming practices (Yang et al., 2020). Thus in recent years, experiments involving long-term cultivation are attracting greater interest because it is possible to follow the changes in soil parameters (Ibrahim et al., 2024). According to Cardoso et al. (2013), soil characteristics with swift response to natural processes or man-made activities are considered good soil health indicators. These include chemical indicators such as total C and N, cation exchange capacity, mineral nutrients and organic matter, physical indicators such as bulk density, porosity, soil texture, moisture and aggregation among others. However, the same authors

DOI: 10.34101/ACTAAGRAR/1/16404

have indicated that most of the above attributes generally have a slower response compared to the biological indicators. Parameters that change slowly, for instance base saturation and cation exchange capacity, are useful soil parameters, but not suitable indicators of tillage impacts on the soil (Juhos et al., 2024).

An important benefit of microbiological indicators is that they are able to respond quickly and sensitively to changes in the environment, with microbial biomass and abundance, microbial activity and microbial taxonomic composition and diversity being the three categories where these indicators lie (Semenov et al., 2025). As such, these indicators are key in assessing the health of the soil due to their sensitivity to management methods (Nunes et al., 2020). For instance, soil ergosterol, glomalin-related soil protein together with Arbuscular mycorrhizal fungi have been mentioned as essential biological soil health indicators, vital for boosting plant absorption of water and nutrients. Seasonal dynamics have been shown by Bhattacharjee et al. (2025) to have a substantial influence on soil biological indicators, with rising summer temperatures promoting all of them, with uncultivated land indicating the highest levels.

Similarly, long-term agricultural management practices affect soil health (Zhang et al., 2024). Among these, continuous use of high dose of chemical fertilizers is responsible for alteration of soil physicochemical and biological properties coupled with a decline in the quality of soil for agricultural purposes (Pahalvi et al., 2021). The degree of soil disturbance caused by tillage operations likewise affects soil biological activity, mostly driven by changes in the organic carbon content of the soil. The size and direction of the changes, relies on several factors which include experimental, inherent as well as the agronomic factors (Nunes et al., 2020). Hence, reducing tillage intensity improves soil organic carbon content, labile C and N fractions of soil organic matter and biological activity (Nunes et al., 2020). Hence maintaining the health of the soil is vital for long-term production of crops in the field (Tu et al., 2021).

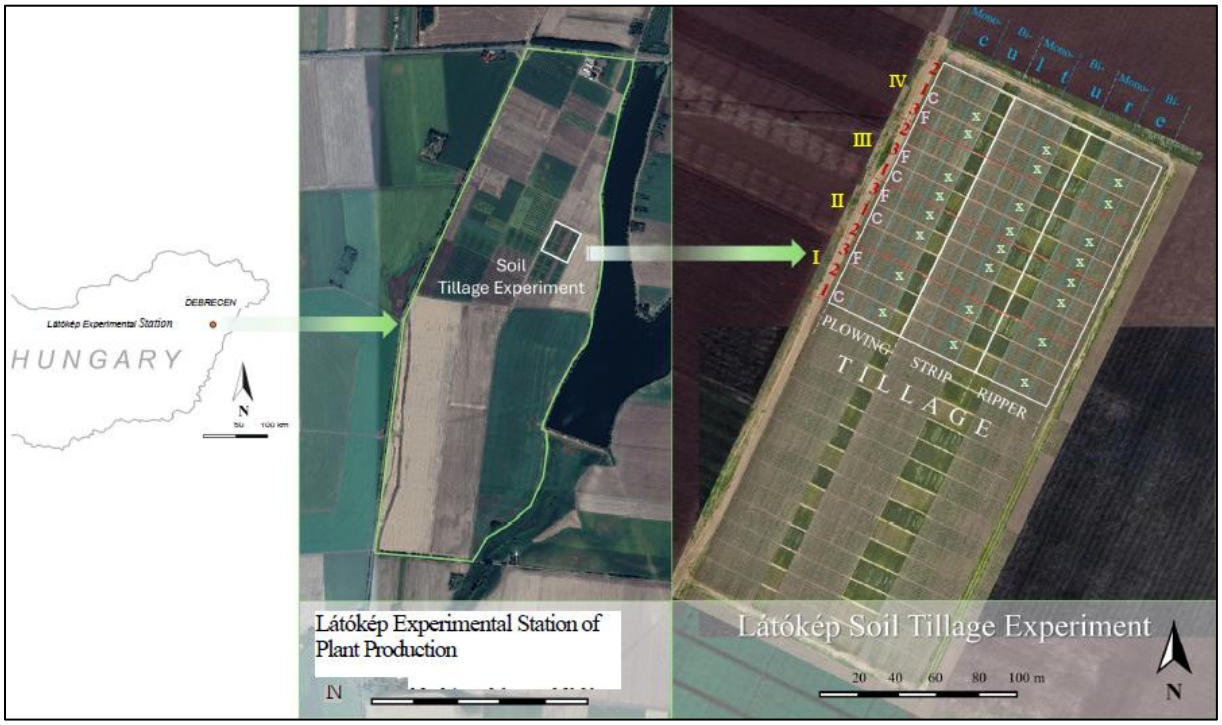
Therefore, due to tremendous influence of agrotechnical interventions on soil microbial activities and biomass, this study aimed at evaluating the impact of long-term tillage and NPK mineral fertilizer application on soil microbial responses in corn under corn-winter wheat rotation.

MATERIALS AND METHODS

Experimental design and sampling procedure

The soil samples analyzed for microbial biomass and community composition were obtained from plots which were selected from the long-term experiment of the University of Debrecen, established in 1991 at Látókép on Endocalcic Chernozem soil (47°33'N, 21°26'E). The polyfactorial experiment included multiple treatments, from which we sampled the following: three tillage systems (Moldboard tillage-MT, Strip tillage-ST and Ripper tillage-RT), NPK fertilizer (composed of 160 kg ha^{-1} N, 60 kg ha^{-1} P_2O_5 and 90 kg ha^{-1} K_2O) and a control (with no fertilization) and a crop rotation of winter wheat and corn (*Figure 1).*

Figure 1. **The long-term experimental set-up at Látókép, 2024**



C= control; F = NPK fertilization; I, II, III, IV = block numbers; x = sample collection points, Biculture = corn-winter wheat rotation

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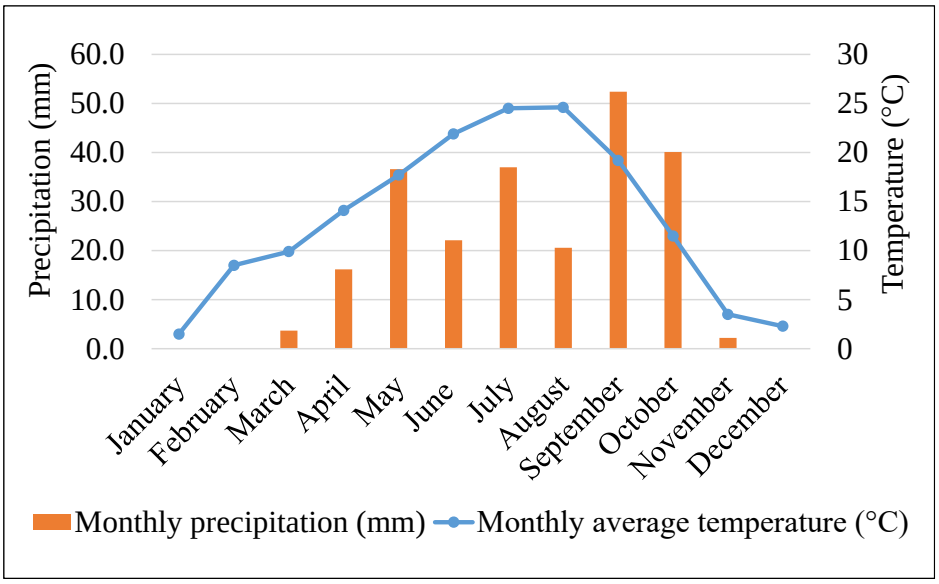
The three tillage systems (MT, ST, RT) differ based on tillage intensity and crop residue management, as described by Karamchand (2021); Balla Kovács et al. (2024) and Kovács et al. (2025). Ripper tillage is a form of reduced tillage practice in which an implement called a ripper is used to break the hard pan in the 30–45 cm soil layer so that the soil is deeply loosened without upturning the soil and residues incorporated into the 0–10 cm upper layer of the soil. In strip tillage, only a narrow band of approximately 25–30 cm is tilled, followed by removal of crop residues from the tilled strip. The soil is worked to a depth of 25–30 cm and only about one-third (33%) of the area is disturbed. This tillage practice retains a high residue cover on the soil surface of about 60–80%. In contrast, moldboard tillage uses a moldboard plow to completely invert the soil in the 0–30 cm layer resulting into much lower residue retention after planting, not exceeding 15% as the residues are incorporated at the plough depth. Therefore, moldboard tillage has the most soil disturbing effect compared to ripper and strip tillages.

In the experiment, each treatment was replicated three times. Soil sub-samples were collected from three randomly selected points in each plot from all the treatments at a plough depth of 0–30 cm on June 7th 2024, as close to the corn plants as possible. The three sub-samples per plot were then composited after thorough mixing by hand. At the time when sampling was done, the corn plants were in their vegetative stage with an average of seven fully expanded leaves. During sampling, the three sub-samples were collected along the 8th corn row in the control and NPK treated plots. The soil samples were subsequently transported to the laboratory for processing before lyophilization.

During the month of June when soil sampling was done, total precipitation (22.1 mm) was lower than in May (36.6 mm). Notably, no precipitation occurred during the five days preceding sampling. In addition, the mean air temperature in June was 4.2 °C higher than in May (*Figure 2*).

Figure 2. **Mean monthly temperature and precipitation for Látókép experimental station, 2024**



Laboratory sample processing

Upon returning from the field, thorough mixing of composite samples was done and then soil was transferred to clean, well labelled glass pots, taking great care to avoid roots and other foreign materials. They were then frozen at -20 °C for 48 h. On the third day, the samples were freeze dried in a lyophilizer for 24 h. The freeze-dried soil samples were immediately disaggregated manually by gently striking with a wooden mallet to reduce particle size and there after transferred into clean, well labelled plastic bottles and securely sealed with screw-top lids. Thereafter they were kept at -20 °C until required for further analysis. The moisture content of the soil was determined by gravimetric method (FAO, 2023) and expressed as the percentage of dry soil:

$$MC = \frac{(Ww - Wd)}{Wd} * 100$$

where *MC* = moisture content in (%), *Ww* = weight of wet soil (g), *Wd* = weight of dry soil

Determination of microbiological parameters of the soil

Phospholipid Fatty Acid (PLFA) analysis using the procedure of Ellis and Ritz (2018) was used to determine the soil microbial biomass. The quantification of the total soil microbial biomass and its community structure (Gram-negative (GN) bacteria, Arbuscular mycorrhizal (AM) fungi, Actinomycetes, Gram-positive (GP) bacteria, saprophytic fungi) was done as indicated in the study carried out by Kovács et al. (2025). The composition of the soil microbial

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community was determined using indicator PLFAs classified into microbial taxonomic groups as follows: *Arbuscular mycorrhizal* fungi as 16:1ω5 PLFA (Frostegård et al., 1993); fungi as 18:2ω6 PLFA (Frostegård et al., 1993); Gram-negative bacteria as monounsaturated and cyclopropyl PLFAs (Zelles, 1999), Gram-positive bacteria as iso- and anteiso-saturated, branched PLFAs (Zelles, 1999); and actinomycetes as 10-methyl fatty acids (10Me16:0, 10Me17:0, 10Me18:0) (Zelles, 1999).

Data analysis

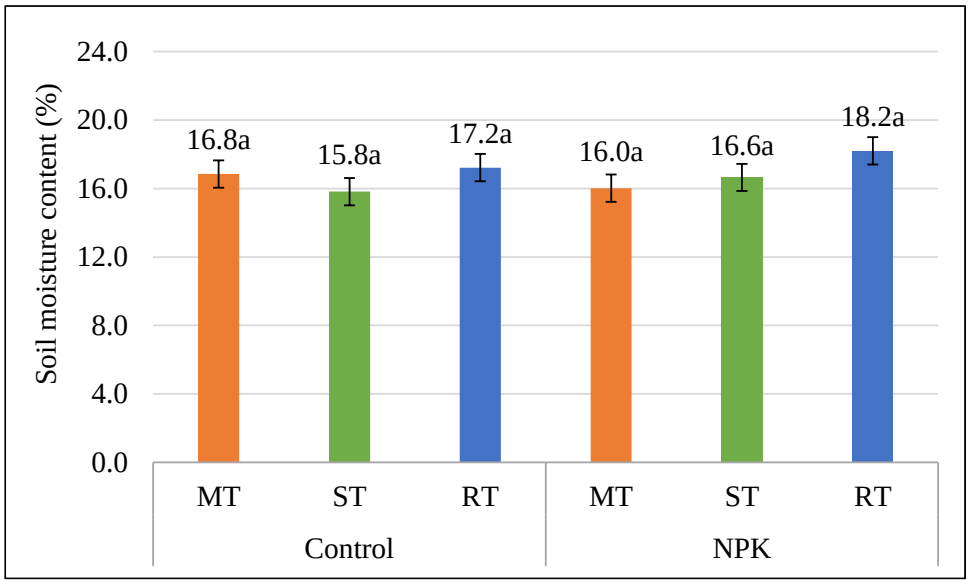
Prior to data analysis, the Levene's test was performed to check for homogeneity of variance. The Shapiro-Wilk test and visual observation of the Q-Q plots was also done to test for normality. Where the data was not normally distributed or variances not homogenous, a log transformation (log10(x)) was applied. The data was then subjected to two-way analysis of variance (ANOVA) to test treatment effects and interactions on soil microbiological parameters, followed by a t-test to compare NPK treatment effect with control within each tillage system and finally a One-way ANOVA to compare tillage systems. Data were analysed using SPSS software (version 29).

RESULTS AND DISCUSSION

Soil moisture content

Results of soil moisture content (*Figure 3*) indicate that ripper tillage (RT) had a slightly higher moisture content than moldboard tillage (MT) and strip tillage (ST), in both the control and NPK fertilized plots. This may partly explain why RT showed a higher soil microbial biomass than other tillage types, as deep soil loosening and shallow incorporation of crop residues (0–10 cm) improved infiltration and water retention. Bekele et al. (2022) reported that conservation tillage practices improve soil infiltration and moisture retention as compared to conventional tillage practices. However, the difference in moisture content in the different tillages and NPK fertilized and control plots was not statistically significant ($p > 0.05$) (*Table 1*).

Figure 3. **Mean soil moisture content (%) in different tillages under NPK and control treatments**



MT = Moldboard tillage; ST = Strip tillage; RT = Ripper tillage. Error bars = Standard error of the mean

Table 1. **Two-way ANOVA showing the effect of tillage, NPK fertilization and their interaction on the soil moisture content (June, 2024)**

Independent variables	Moisture content (%)
Tillage	0.173ns
NPK fertilization	0.623ns
Tillage x NPK	0.473ns

ns = non-significant at 5% level

Soil microbial biomass and community composition

Results of a two-way ANOVA (*Table 2*) indicate that all microbiological parameters were significantly affected by tillage ($p \leq 0.05$). However, NPK fertilization significantly influenced only Arbuscular mycorrhizal fungi ($p = 0.000$), Gram-negative bacteria ($p = 0.046$) and actinomycetes ($p = 0.036$). No statistically significant interaction effect was observed between tillage and NPK fertilization in almost all the microbiological parameters. The exception was observed in Arbuscular mycorrhizal fungi, where the interaction was significant.

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Table 2. **Two-way ANOVA showing NPK fertilization, tillage and their interaction effects on the soil microbiological parameters (June, 2024)**

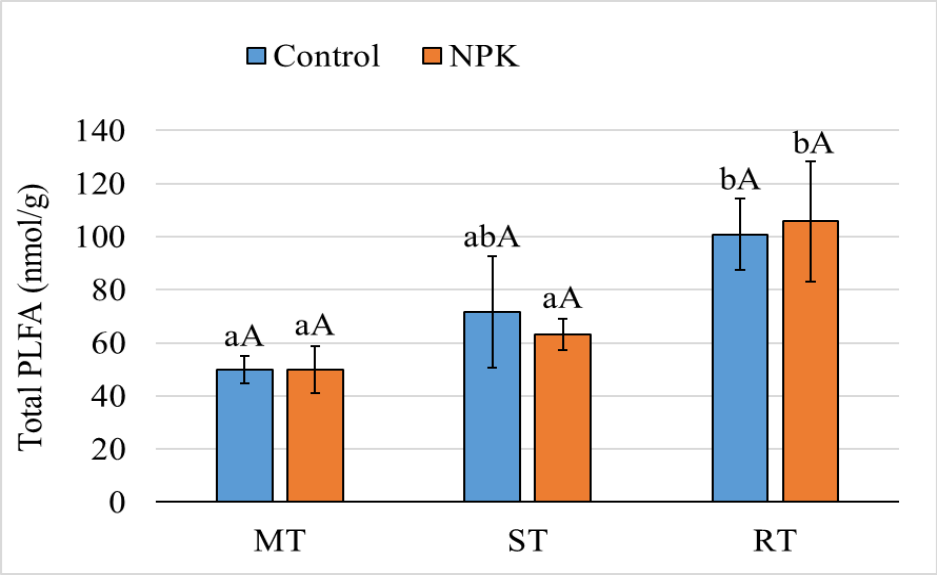
	Microbiological parameters					
Independent variables	**Total PLFA**	**GN bacteria**	**Fungi**	**GP bacteria**	**AM fungi**	**Actinomycetes**
Tillage	0.000***	0.000***	0.000***	0.000***	0.000***	0.003**
NPK fertilization	0.839ns	0.046*	0.069ns	0.815ns	0.000***	0.036*
Tillage x NPK	0.663ns	0.184ns	0.086ns	0.371ns	0.010**	0.161ns

****p* ≤ *0.001; **p* ≤ *0.01*; *p* ≤ 0.05; *ns = non-significant*

The soil microbial biomass

Results in *Figure 4* indicate that total PLFA biomass, an indicator of soil microbial biomass was strongly affected by tillage and to a lesser extent by NPK fertilization. There was no significant difference in microbial biomass between fertilized (42.7–125.7 nmol g^{-1} soil) and control plots (44.7–110.1 nmol g^{-1} soil). The microbial biomass increased with decreasing tillage intensity, with the highest biomass observed in RT (87–126 nmol g^{-1} soil) followed by ST (51–83 nmol g^{-1} soil) and the least in MT (43–58 nmol g^{-1} soil) across NPK fertilized plots and control plots. This result agrees with Zhang et al. (2013) who reported a generally higher abundance of microbes in the conservation tillages than in conventional tillage. In this study, the microbial biomass did not significantly differ between the control plots (45–110 nmol g^{-1} soil) and NPK fertilized plots (43–126 nmol g^{-1} soil) within the tillage types. On the contrary, Balla Kovács et al. (2024) reported that NPK fertilization decreased the total PLFA, however, in their study soil sampling and measurements were conducted in autumn, whereas in the present study they were performed in early June.

Figure 4. **Tillage and NPK fertilization effect on soil microbial biomass**



Uppercase letters that are different denote significant differences between NPK and control treatments within the same tillage system; lowercase letters that are different denote significant differences across tillage systems within the same treatment; MT = Moldboard tillage; ST = Strip tillage; RT = Ripper tillage; Error bars = 2 x Standard error of the mean

Soil microbial community composition

Generally, the composition of the soil microbial community was greatly influenced by tillage and to a lesser extent by NPK fertilization (*Figure 5*).

Arbuscular Mycorrhizal fungi (AM fungi): NPK fertilization negatively affected the biomass of AM fungi, as generally lower biomass was observed in NPK fertilized plots (1.0–2.9 nmol g^{-1} soil) than control plots (2.2–11.7 nmol g^{-1} soil) (*Figure 5A*). This result agrees with findings of Gryndler et al. (2006); Ma et al. (2021), and Kovács et al. (2025) that have also indicated in their studies a significantly lower AM fungal biomass in NPK fertilized than control plots. For instance, measurements of the concentrations of the fatty acid 16:1ω5, a biomarker of AM fungi in fertilization experiment indicated that application of inorganic fertilizers reduced significantly the concentration of the 16:1ω5 fatty acid in soil irrespective of sampling season (Gryndler et al., 2006). The authors reported reduced AM fungal growth and root colonization following NPK mineral fertilizer application. Additionally, Ma et al. (2021) showed in their experiment that prolonged NPK fertilizer addition to the soil resulted in a decline of AM fungal biomass in

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wheat rhizosphere. These studies attributed this decline to alteration of soil properties as a result of long-term fertilization, particularly the rhizosphere soil pH and available phosphorus level. Either low pH or high phosphorus availability reduces the colonization of AM fungi which consequently results in decline of the AM fungal biomass. Similarly, Ullah et al. (2019) observed a significant reduction of AM fungi abundance after 9 years of continuous N fertilization. The reduced AM fungi abundance was attributed to reduced soil pH. Soil acidification has been reported by Ullah et al. (2019) to affect plant nutrient availability and inhibit soil organic matter decomposition.

Likewise, tillage had a significant effect on AM fungi, with the highest biomass observed under RT both in the control (mean = 9.6 nmol g^{-1} soil) and NPK treated plots (mean = 2.3 nmol g^{-1} soil). The highest AM fungi biomass in RT could be due to the less disruptive environment that is created by RT, which is a reduced tillage practice as compared to the more disruptive conventional MT. Galvez et al. (2001) reported higher colonization of maize roots and spore populations in reduced tillage than in conventional tillages (moldboard plowed or chisel-disked soil). This therefore implies that reduced tillage is more beneficial to AM fungi than conventional tillage types. Soil disturbance has been reported by Hart and Reader (2004) to affect soil hyphal length, fungal biomass and colonization of new roots, with greater disturbance in AM fungi that rely on living mycelia for colonization since they are likely to be damaged by soil disturbance than spores.

Fungi: NPK fertilization did not have a significant effect on the biomass of saprophytic fungi within all the tillage types (*Figure 5B*). Our result agrees with Gryndler et al. (2006); Marschner et al. (2003). Gryndler et al. (2006) observed that the whole cell fatty acid 18:2ω6,9, a biomarker fatty acid of saprophytic fungi was not affected by inorganic fertilization while Marschner et al. (2003), reported in their long-term fertilization experiment that the biomass of soil fungi measured as 18:2ω6 did not respond to different fertilization regimes (both organic and inorganic soil amendments). It has hence been suggested that while other soil microbes are affected by fertilization (mineral and organic), saprophytic fungi seem to be almost unaffected by such treatments (Gryndler et al., 2006). Tillage on the contrary had a great influence, with the highest fungi biomass recorded under RT (2.3–10.0 nmol g^{-1}soil) and the lowest under MT (1.0–1.3 nmol g^{-1} soil) in both NPK treated and control plots. This result agrees with findings of Kovács et al. (2025), from samples collected in 2023 from the long-term experiment, which showed that the saprophytic fungal biomass was greatly influenced by tillage, with a higher fungi biomass in reduced tillage types than in conventional tillage. The authors attributed the higher fungal biomass to higher soil organic matter content associated with reduced tillage types. Additionally, reduced tillage practices have been reported by Sándor et al. (2020) to be less disruptive to fungi than conventional tillage practices, hence creating a nutrient rich environment suitable for multiplication and activity of fungi.

Bacteria (*GN bacteria* and *GP bacteria*): NPK fertilization did not change either Gram-negative bacteria or Gram-positive bacteria biomass significantly within all tillage types (*Figure 5C* and *5D*). However, tillage led to a significant shift in the biomass of both microbial groups in NPK treated and control plots. The highest biomass was recorded under RT followed by ST and the least under MT. This implies that reduced tillages (Ripper tillage and Strip tillage) provide a more conducive environment for bacteria than the conventional moldboard tillage. This is possibly due to enhanced residue retention and organic matter accumulation in RT and ST, which provide stable substrates and microhabitats for microbial proliferation. Zhang et al. (2018) reported that conservation tillage practices when coupled with straw returning can increase organic carbon accumulation and alter microbial community. Gram-negative bacteria showed a generally lower biomass ((RT (15.8–23.9 nmol g^{-1} soil), ST (10.4–20.1 nmol g^{-1} soil), MT (8.6–11.9 nmol g^{-1} soil)) than Gram-positive bacteria ((27.3–42.7 nmol g^{-1} soil), ST (17.0–28.0 nmol g^{-1} soil), MT (14.5–22.3 nmol g^{-1} soil)) under all tillages. Moreover, the effect was more pronounced in the conventional tillage type (MT) than the reduced tillages (RT and ST). Kovács et al. (2025) made a similar observation. The lower biomass of Gram-negative bacteria could be due to their sensitivity to stresses resulting from environmental disturbances, unlike Gram-positive bacteria that are more resilient due to their structural and physiological characteristics.

Actinomycetes: The actinomycetes biomass was significantly affected by NPK fertilization. The biomass tended to increase, particularly under MT and RT in plots where NPK fertilizer was applied (*Figure 5E*). This shows that NPK fertilization increases actinomycetes biomass. Results from a meta-analysis conducted by Jiangwei et al. (2020) indicated that NP and NPK addition to the soil increased actinomycetes in crop lands. Subhashini and Kumar (2019), in their long-term manure experiment on tobacco similarly showed an increase in the biomass of actinomycetes in treatments where mineral fertilizers containing N, P and K were applied singly or combined, in comparison to plots where no external inputs were applied. A comparable trend was reported by Kovács et al. (2025), who observed an increase in actinomycetes biomass in NPK fertilized plots than control plots. Bairwa et al. (2021) likewise reported increased actinomycetes counts in NPK treatments than the control in soybean-wheat rotation.

It can also be seen from this experiment that tillage significantly affected the biomass of actinomycetes, with least effect observed under RT in NPK fertilized plots than in ST and MT.

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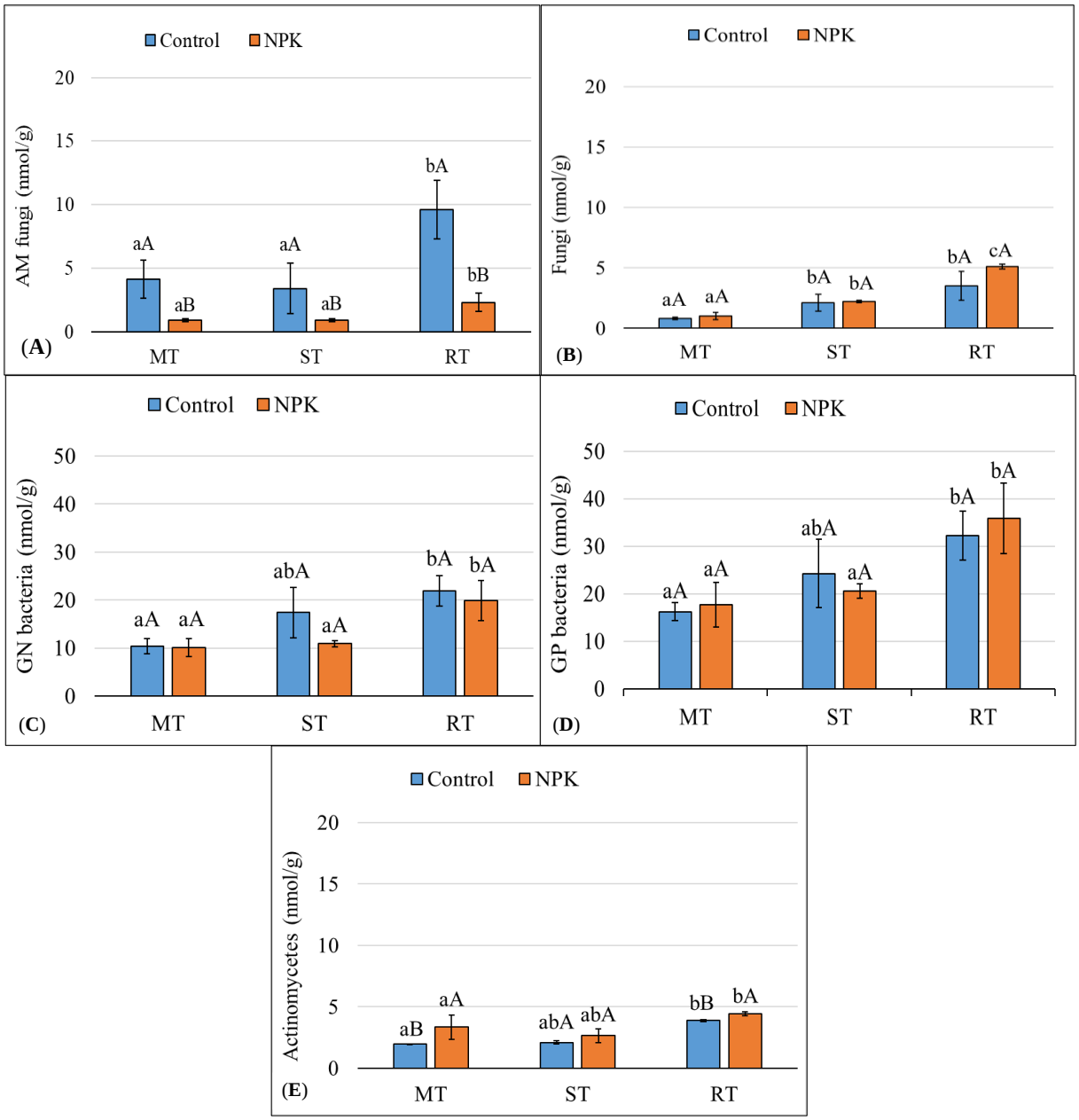
Figure 5. **Tillage and NPK fertilization effect on soil microbial community composition**



Uppercase letters that are different denote significant differences between NPK and control treatments within the same tillage system; lowercase letters that are different denote significant differences across tillage systems within the same treatment. MT = Moldboard tillage; ST = Strip tillage; RT = Ripper tillage; (A) *Arbuscular mycorrhizal* fungi; (B) Fungi; (C) Gram-negative bacteria; (D) Gram-positive bacteria; (E) Actinomycetes; Error bars = 2 x Standard error of the mean

CONCLUSIONS

The study evaluated the impact of long-term tillage practices and NPK mineral fertilizer application on the soil microbiome in corn grown under corn-winter wheat rotation. Tillage generally had a stronger effect on the soil microbiological parameters than NPK mineral fertilizer application, with the highest microbial biomass and community observed under ripper tillage (RT) and the least under moldboard tillage (MT). In terms of microbial biomass abundance, GP bacteria and GN bacteria were the most abundant, followed by *Arbuscular mycorrhizal* (AM) fungi, whereas saprophytic fungi and actinomycetes recorded the least abundance. It can therefore be concluded from this experiment that reduced tillages were more favorable than the traditional moldboard tillage.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

DOI: 10.34101/ACTAAGRAR/1/16404

ACKNOWLEDGEMENTS

The authors are grateful to Professor János Nagy, founder of the long-term field experiment at Látókép, and to the Institute of Land Use, Technology and Precision Technology for making the measurements possible.

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