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Changes in metabolic characteristics of soil microbial communities during intercropping of oats and vetch

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Abstract

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The excessive use of chemicals and monoculture cultivation has led to damage to soil and plant health, which is also a consequence of the reduced diversity of the soil microbiome. Various approaches related to intercropping, reduced tillage practices and organic amendments are applied to restore biodiversity in agroecosystems. This study aims to illuminate the changes in soil microbiome metabolic potential during the intercropping of oats and vetch. Vetch and oats seeds were sown at 3:1 proportion in spring. Soil samples were taken four times: before sowing; from the rhizosphere during the tillering and ripening phases; and 2 months after incorporation of the plants at a depth of 20 cm into the soil by ploughing, as green manure. We found the highest microbial activity as soil basal and induced respiration, as well as the activity of soil dehydrogenase enzyme in the ripening stage. In contrast, the highest level of metabolization of carbon compounds of the Ecoplates was found in the tillering one, followed by ripening, green manure phase, and before sowing. The strongest uptake by microbial communities was observed for amino acids, polymers, carboxylic acids and carbohydrates. Differences in community-level physiological profiles (CLPP) of the rhizosphere microbiome were discovered using principal component analysis. P.C. 1 of 62.69% showed the highest correlation of L-arginine and α-cyclodextrin before sowing, β-methyl-D-glucoside, and D-glucosaminic acid during tillering, α-D-lactose, and L-asparagine during tillering and ripening, and of L-threonine, glycogen, and D-glucosaminic acid in case of green manure. PC2 contributed 18.47%, and was primarily associated with amino acids and carbohydrates. Because increased microbial metabolic capacity has a positive effect on soil processes, this study should be considered very useful in evaluating the development of soil communities during the stages of oats and vetch intercropping.

Keywords: CLPP; microbial activity; intercropping; oats; vetch

Introduction

Overusing soils in the last few years have significantly impacted agroecosystems. In many sites, the monoculture cropping and intensive application of synthetic fertilizers, resulted in exhausting and loss of main nutrient content, and decreased soil microbial functionality dramatically (Tsiafouli et al., 2015; Dubey et al., 2019). According to plenty of sources, the application of diversified cropping, or even the joint cultivation of two crops or intercropping, dramatically contributes to improved crop performance and soil health (Li et al., 2023; Qu et al., 2022; Fitouri et al., 2022). In that sense, authors reported changes in crop performance yield and quality by applying different proportions of oat and vetch during intercropping at 3:1 (Qu et al., 2022). That is partially due to the increased soil microbial diversity in such systems (Chinthalapudi et al., 2023).

The study of the soil-microbiome-plant system allows for acquiring important information about the transformation, the quantitative and qualitative changes of the organic matter in the soil, the microbiological activity and biomass, and the metabolic profiling at the community level, but also about the soil structure, the dynamics of organic elements in soil and plant production. Parameters, such as soil respiration, dehydrogenase enzyme activity and the microbial community's metabolic profile, can be used for soil quality diagnosis and comparison between different soil types (Shrestha et al., 2015; Gaublomme et al., 2006; Poonia et al., 2024; Irving et al., 2024). Soil microbiome is often used to predict future changes in the soil nutrient balance, because they respond rapidly to any changes due to their fast metabolism (Shilev et al., 2019). As they are in immediate contact with the active part of organic matter, they participate in diverse soil ecological services. In this sense, any fluctuations in soil properties (crop kind or variety, fertilizer application, tillage practices, etc.) related to soil management, are reflected in the community structure of the soil microbiome (Gomez et al., 2006). Moreover, Chinthalapudi and coworkers concluded, based on the CLPP study, that the crop type substantially affects the soil microbiome's functional diversity. They observed a higher impact of intercropping than the individual crop application (Chinthalapudi et al., 2023). In this sense, we found a gap related to the existence of considerable research information in the literature about soil microbial community changes during intercropping.

In the present study, we aim to reveal the changes in soil microbiome metabolic potential during the intercropping of oats and vetch.

Materials and Methods

The field experiment took place at the training and experimental field of Agricultural University – Plovdiv on alluvial-meadow soil (42°08′15.4″ N 24°48′16.4″ E). International classification of FAO describes them as Mollic fluvisol. After maize cultivation, the oats-vetch mixture was sown on March 2022, at 0,2 ha using *Avena sativa* L. Max cultivar and vetch *Vicia sativa* Obrazets 666 cultivar. We applied 220 kg seeds/ha (3:1 vetch:oats). The mixed cover crops were incorporated into the soil at the ripening stage of oats. Soil samples for soil and microbial analysis were taken before sowing, during the tillering and ripening stage of oats development (from the rhizosphere), and two months after green manure application from the upper soil layer (0–20 cm). The samples were stored in a fridge for several days before processing the analyses.

Soil pH and electrical conductivity (E.C.) were assessed through standard methods of analysis (Thomas, 1996; Rhoades, 1996). The available nitrogen, phosphorus, and potassium were evaluated using the method of Egnér-Riehm (Egnér et al., 1960). Soil organic C was calculated from the organic matter assessed using the potassium dichromate method (ISO 14235:1998).

Basal (B.R.) and induced (SIR) soil respiration were carried out, using the method described by Alef (Alef, 1995b). The metabolic quotient was calculated as a ratio of basal respiration and microbial biomass. For that purpose, the metabolic quotient (M.Q.) was calculated as a ratio of B.R. to soil microbial biomass C (SMB), as described by Anderson and Domsch (1989).

Soil enzyme dehydrogenase activity in studied samples was assessed using classical method of Thalmann (Thalmann, 1968), modified by Alef (Alef, 1995a). It is based on the reduction of tri-phenyl tetrazolium chloride (TTC) to thriphenyl formazan (TPF) by microorganisms. The evaluation of colour change was measuredspectrophotometrically at 546 nm.

The soil microbial physiological profile structure was studied using Ecoplates® Biolog™ system, described in previous studies (Shilev et al., 2024; Insam and Goberna, 2008).

All analyses were carried out in four replicates. One-way analysis of variance (ANOVA) was used for variables at a probability level of $p \leq 0.05$. Principal component analysis (PCA) was applied to describe physiological utilization patterns per each stage. The significance of differences between the means was assessed through the least significant difference (LSD) test. Results were presented after calculating the mean and standard errors using Microsoft Excel v.14.s

Results and Discussion

Table 1 presents the main physicochemical soil properties before sowing, and at the ripening stage of the plants. An increase in pH values was observed at the milk maturity of oats compared to those before sowing, while reducing the electrical conductivity and availability of the investigated elements. This is related, on the one hand, to the assimilation of some of them by the plants and, on the other hand, to the reduced mobility due to the increased values of the soil reaction.

The soil reaction slightly increased during the plant vegetation (Table 2), reaching 8.22 in the ripening stage and 8.41 after the green manure. At the same time, the E.C. decreased from 189.30 $mS.cm^{-1}$ to 116.46 $mS.cm^{-1}$ in the green manure stage. In addition, B.R. indicates the current microbial activity at the time of sampling, while SIR is the potential capacity of each microbiome if a readily usable C-source is supplied. Some researchers, such as Epelde et al. (2008), also use the B.R. and microbial biomass ratio as a general characteristic of microbial activity, called metabolic coefficient. Higher B.R. and SIR were found in the ripening stage of vegetation, where the organic compound excretion from the roots is

Table 1. Soil physicochemical characteristics at layer 0-20 cm

	Clay (%)	pH (H_2O)	EC ($\mu S/cm^{-1}$)	Total N (mg/kg)	N-NH_4 (mg/kg)	N-NO_3 (mg/kg)	Total P (P_2O_5, mg/kg)	Total K (K_2O, mg/kg)	Organic C (g/kg)
Before sowing	31.25	7.78	185.30	20.74	12.44	8.30	3.64	150.20	11.21
Ripening		8.28	126.34	14.93	8.57	6.36	2.8	96.80	12.82

Source: Authors' own elaboration

highly expressed. This trend continued through the growing season, probably due to root exudations in mixed cropping compared to monoculture. In both fields, increased SIR values were observed due to mobilization of the microflora by adding readily digestible carbohydrates to the soil (Fitouri et al., 2022).

We studied the activity of dehydrogenase enzyme with the purpose to describe overall impact of external influences on soil microbes (Table 2). The enzyme is characteristic for interventions in soil as addition of toxic substances or any nutrient supply from external source. It is an extracellular enzyme and is participating in the phosphorylation processes of organics degradation (Trevors, 1984). We found much higher dehydrogenase activity (DHA) during plant vegetation, probably due to the increased general microbial activity and availability of organic compounds for degradation (Usmani et al., 2019). The rate was much higher in ripening stage followed by the tillering one. These data correlated with the general microbial activity as soil respiration. Lower DHA was detected before sowing. The existence of newly added organic substrate, such as the plant tissues after green manuring, was reflected in relative increase in activity of this enzyme compared to the control.

Ecoplates are a very suitable and widespread approach to characterize the changes occurring in the soil. Still, at the same time, it allows a detailed presentation of the ability of the soil microbiome to use the diverse 31 carbon sources (Garland and Mills, 1991). In this metabolic study, the utilization of the C-sources increased over time. The lowest total microbial activity and ability to metabolize the organic matter was found before sowing (Fig. 1), which was explained by the absence of plants and a reduced population, quantitatively, and in terms of species abundance. The tillering stage showed a substantial increase in the carbon assimilation capacity by the communities, increasing the utilization capacity at 168 h from an optical density of 1.3 before sowing to 1.85 in tillering. In the ripening phase, uptake rates were within the standard error with the tillering, although the ripening activity was higher during the first half of the study (0–96 h). After green fertilization, a slight decrease in metabolic capacity was observed, which was higher than control (before sowing). Researchers also reported about the diverse influence of fertilization or type of crop on metabolic activity of soil communities (Chinthalapudi et al., 2023; Khalifa et al., 2022).

Figure 2 shows the soil microbiome's ability to assimilate each studied organic compound group. Amino acids are

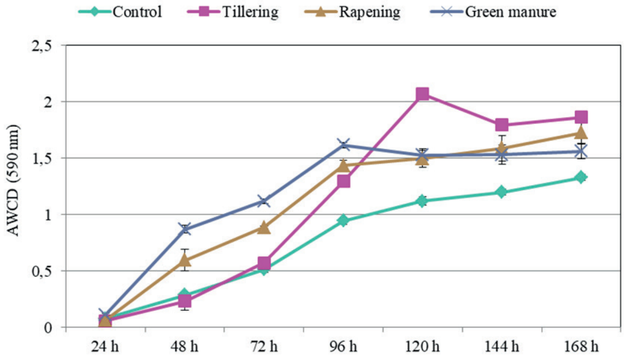

Fig. 1. Changes in average well color development over time of soil microbial communities at 590 nm (left) Data represent the means and the standard errors (n = 3)

Source: Authors' own elaboration

Table 2. Soil rhizosphere physicochemical and microbial properties during the plant stages

	pH	EC ($\mu S/cm^{-1}$)	CO_2 ($\mu g\ g^{-1}h^{-1}$)		MQ	DHA
			BR	SIR		
Control	7.78c	189.30a	41.0b	46.1c	22.25a	0.98d
Tillering	7.82c	145.11b	34.7c	45.2c	19.17b	1.73b
Ripening	8.22b	126.34c	46.6a	53.9a	21.65a	2.61a
Green manure	8.41a	116.46d	33.6c	44.8c	18.75b	1.13c

The results showed the mean of five replicates. Different letters indicate statistically significant differences between stages at significance of $p \leq 0.05$. Control – before sowing; BR – basal respiration; SIR – substrate-induced respiration; MQ – metabolic quotient; DHA – dehydrogenase activity.

Source: Authors' own elaboration

the most preferred source by the soil communities, followed by polymers, carbohydrates, and carboxylic acids. Similarly, researchers found a high response of microbial communities to adding mineral and combined (mineral and organic) fertilization (Chinthalapudi et al., 2023; Bulluck et al., 2002). At the same time, the control treatment was associated with the dominance of polymer-utilizing communities (Shilev et al., 2024). In another case, increased AWCD after mineral or organic fertilization was also reported. Higher discrepancy between rhizosphere and non-rhizosphere treatments was found instead between fertilization types (Zhang et al., 2020). Of interest are the dominant metabolic profiles during the different phases. Before sowing (control), the soil microbiome utilized primarily amino acids and carboxylic acids. When plants were in the tillering phase, the microbiome increased its capacity to utilize all the studied compounds but especially polymers, amines and amides, and carbohydrates by 82.1%, 55.7%, and 50.75%, respectively. The tillering was followed by a significant statistically proven activity increase of communities, utilizing phenolic compounds. The phenolics may be secreted by the plant roots, soil lichates, produced by microorganisms, or because of organic decomposition (Nowak et al., 2022; Lochab et al., 2014). They could be utilized as sole C-source by different bacteria, such as *Alcaligenes* sp., *Pseudomonas* sp., *Paenebacillus* sp., *Arthrobacter scleromae, Rhodococcus aetherivorans* and Rh. *pyridinivorans*, *Bacillus* sp., *Glutamicibacter nicotianae*, among others (Thavamani et al., 2012; Nowak et al., 2022). It seems tillering phase was very stimulating for the utilization of whole studied organic compounds by the microbial communities, while in the absence of plants, the activity was lowest. Similar findings were reported by Zhang and collaborators comparing diverse fertilization and CLPP in rhizosphere and non – rhizosphere (Zhang et al., 2020). As a leguminous crop, vetch demonstrated a low C:N ratio, and should be rapidly degraded in soil, increasing soil nitrogen availability (Chinthalapudi et al., 2023). The expected consequence of this rinse nutrient supply should be the activation of microbiome activity that was not seen in our study (Fig. 1). After the green fertilization, highest decrease was observed in case of the carboxylic acid-consuming communities, by 32% as well.

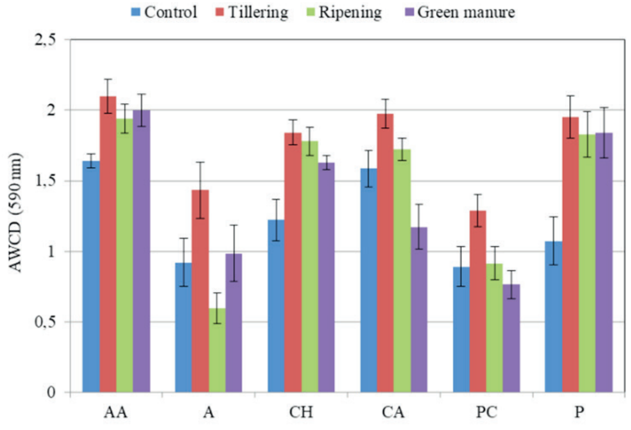

Fig. 2. Average well-colour development of different groups of C-substrates
Data represent the means and the standard errors (n = 3). AA: Amino acids; A: amines and amides; CH: carbohydrates; CA: carboxylic acids; PC: polyphenols; P: polymers.
Source: Authors' own elaboration

The amino acids are very important source of carbon, nitrogen and energy for soil bacterial communities (Halvorson, 1972). Previous investigations showed that they are preferable compounds for most species, including *Escherichia coli, B. subtilis*, *Pseudomonas aeruginosa, Salmonella typhimurium.* Some authors reported that *E.coli* and *S. thyphimurium* prefer L-amino acids, such as L-alanine, glycine, L-asparagine, L-cysteine, L-glutamate, and L-serine (Hedblom and Adler, 1983), while *B. subtilis* absorb all twenty commonly found amino acids (Ordal and Gibson, 1977). In our study, the amino acids were the organic substances, used the most by soil microbial communities (Fig. 3A). During the vegetation of the crops, a succession was observed, and during the tillering stage, the L-arginine and L-asparagine assimilating communities prevailed. In contrast, the glycyl-L-glutamic acid utilizers had a significant influence during ripening. The rest of the amino acids were primarily consumed during the green manure stage. The group of amimes and amides were consumed less than most organic compounds (Fig. 3B). Nevertheless, they were used mainly during the tillering stage. The carbohydrates were highly utilized compounds by the microbial communities (Fig. 3C). The communities utilizing D-cellobiose, α-D-lactose, β-Methyl-D-glucoside, and N-acetyl-D-glucosamine during the tillering stage of oats were dominant. D-mannitol and glucose-1-phosphate were equally consumed during plant vegetation.

Regarding carboxylic acids, their uptake is multidirectional (Fig. 3D). For γ-hydroxybutyric acid and D-glucosaminic acid, no changes were found in the different phases of plant development, while for the rest, changes in the degree of absorption were observed during the various stages. The itaconic, D-malic, and D-galacturonic acids were consumed intensively by the soil microbiome during tillering and ripening stages. In opposite, α-ketobutyric acid' absorption was decreased during both vegetation stages, while pyruvic acid methyl ester was utilized mostly before sowing and during tillering. The phenolic compounds are consumed traditionally from a minor part of the soil communities (Fig. 3E). Thus, 4-hydroxy benzoic acid was consumed relatively well, especially in the tillering and ripening stages. The microbial communities utilized the polymers in similar magnitude except for tween compounds before sowing (Fig. 3F). A Higher rate took α-cyclodextrin during the tillering stage.

Recent results in soybeans and cover crops including common vetch showed that carboxylic acids, amino acids, and carbohydrates are responsible for soil communities' metabolic diversity, with 46.4% of the variance (Chinthalapudi et al., 2023). In addition, they conferred that functional diversity is also associated with pH changes, while total carbon and total nitrogen concentration demonstrated a negative correlation.

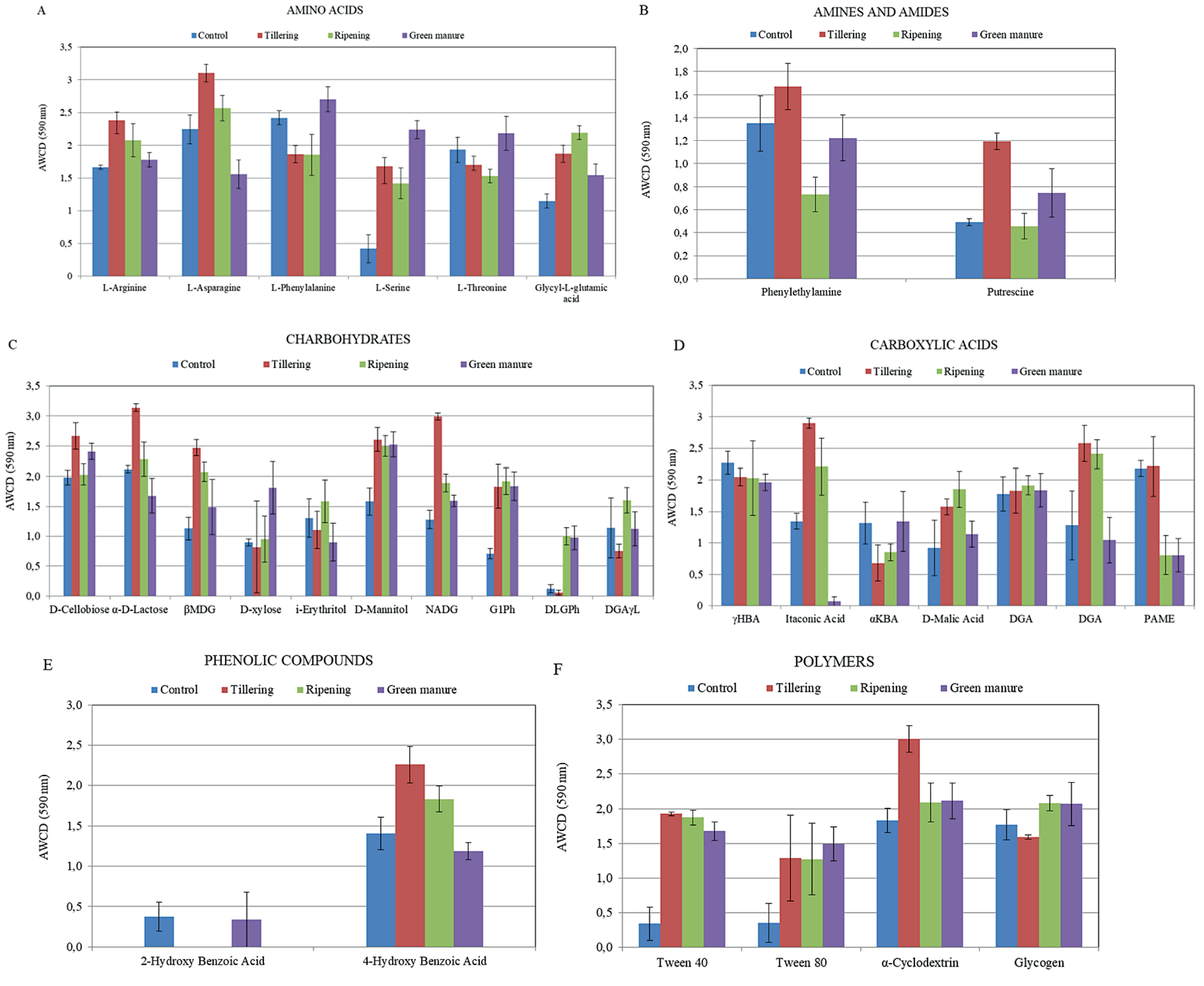

Fig. 3. AWCD of each one of the 31 C-substrates of the Ecoplates at 590 nm
The results show the means and the standard errors (n = 3). A: amino acids, B: amines and amides, C: carbohydrates, D: carboxylic acids, E: phenolic compounds, F: polymers.

Abbreviations: βMDG: β-Methyl-DGlucoside, NADG: N-Acetyl-DGlucosamine, G1Ph: Glucose-1-Phosphate, DLGPh: D,L-α-Glycerol Phosphate, DGAγL: D-Galactonic acid γ-Lactone, γHBA: γ-Hydroxybutyric acid, PAME: Pyruvic Acid Methyl Ester, DGA: D-galacturonic acid, DGA: D-Glucosaminic acid; αKBA: α-Ketobutyric acid

Source: Authors' own elaboration

Soil elements' concentration, forms and availability, stresses, plant species or variety, and many more factors affect soil microbiome development. During the different stages of their growth, plants have different nutritional requirements. Still, at the same time, they release diverse organic substances through the roots and change the structure of the microbial communities in the soil (Shilev et al., 2019; 2022). Figure 4 shows a biplot in the first and second principal components (P.C.) dimensions in the studied soil samples of oat-vetch intercropping (before sowing, tillering, ripening, green manuring). Compounds of various groups, such as glycogen, D-cellobiose, D-mannitol, D-glucosaminic acid, γ-hydroxybutyric acid L-phenylalanine, and L-threonine had positive effects for the first and second P.C. The highest absorption rate of any treatment in the experiment distinguished these two carbon groups. PC1 contributed 62.69% to the total variation and correlated strongly with amino acids, carboxylic acids, carbohydrates, and polymers. PC2 contributed 18.47%, and was associated chiefly with amino acids and carbohydrates. The highlighted findings are confirmed by the results of Chinthalapudi and co-authors, establishing highest utilization of amino acids, carboxylic acids and carbohydrates during their investigation (Chinthalapudi et al., 2023). The lowest metabolization rate was measured for 2-hydroxy benzoic acid and putrescine, with negative values for both PCs. The highest correlation between the ripening and tillering stage of plant development was found for α-D-lactose, L-asparagine, and glycyl-L-glutamic acid.

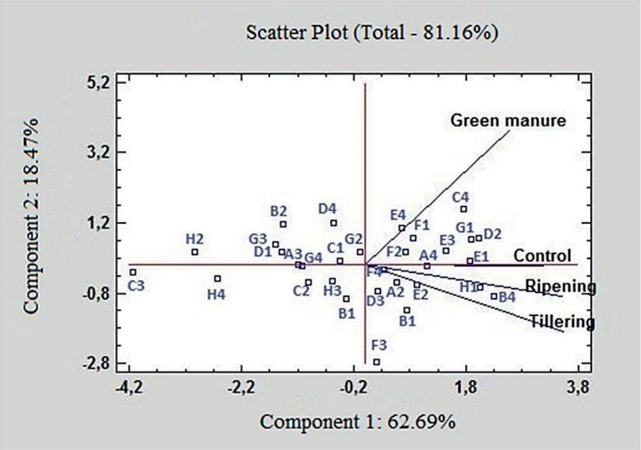

Fig. 4. PCA ordinations of different carbon sources utilization (indicated by AWCD) of Ecoplates at 590 nm

C1: tween 40, D1: tween 80, E1: α-cyclodextrin, F1: glycogen, G1: D-cellobiose, H1: α-D-lactose, A2: β-methyl-Dglucoside, B2: D-xylose, C2: i-erythritol, D2: D-mannitol, E2: N-acetyl-Dglucosamine, G2: glucose-1-phosphate, H2: D,L-α-glycerol phosphate, A3: D-galactonic acid γ-lactone, C3: 2-hydroxy benzoic acid, D3: 4-hydroxy benzoic acid, E3: γ-hydroxybutyric acid, F3: itaconic acid, G3: α-ketobutyric acid, H3: D-malic acid, F2: D-glucosaminic acid, B3: D-galacturonic acid, B1: pyruvic acid methyl ester, A4: L-arginine, B4: L-asparagine, C4: L-phenylalanine, D4: L-serine, E4: L-threonine, F4: glycyl-L-glutamic acid, G4: phenylethylamine, H4: putrescine.

Source: Authors' own elaboration

Conclusions

The physiological profiles of soil microbiome in intercropping oats and vetch vary during vegetation exhibiting a diverse catabolic responses. AWCD showed decreasing C-source utilization capacity of microbiome in studied samples in next order: tillering, ripening, green manure, before sowing. Highest metabolic capacity of microbial communities was found for utilization of amino acids, polymers, carboxylic acids and charbohydrates. PC1 showed highest correlation of C-source metabolization with 62.69%, especially of L-arginine and α-cyclodextrin before sowing, of β-methyl-Dglucoside, and N-acetyl-Dglucosamine during tillering, of α-D-lactose and L-asparagine during tillering and ripening, and of L-threonine, glycogen, and D-glucosaminic acid in case of green manure. The highest microbial basal and induced respiration and dehydrogenase activity were found in the ripening stage. The study of physiological profiles of microbial communities should be combined with classical or genomic approaches, in order to evaluate more in-depth the effect of intercropping on microbiome development.

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Conflict of interest

The authors of the study declare the absence of a conflict of interest.

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