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Application of the Gini coefficient for assessment of microbial communities` diversity in soil treated with biofertilizer

Приложение на коефициента на Джини за оценка на разнообразието на микробните съобщества в почва третирана с биопрепарат

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ABSTRACT

Metabolic activity and structure of microbial communities are important indicators for soil health and fertility. BIOLOG`s Ecoplate patented technology provides a method for estimating soil microbial community structure based on different substrate utilization. The optical density (OD) in the wells changed during the plate incubation due to the reduction of the tetrazolium dye and the OD values are used for the calculation of different indexes as parameters for microbial activity and community structure. The Gini coefficient, which was originally employed by economists for the estimation of wealth and income, has applications in a variety of biological studies. The Gini coefficient, alone or in combination with other indexes, can be used for the assessment of microbial diversity and species evenness. The aim of the current study was to calculate the Gini coefficient at several consecutive time points during the EcoPlate incubation, to make a graphical presentation through the corresponding Lorenz curves and to estimate the effect of applied biofertilizer on the soil microbial diversity. The results showed that the Gini coefficient decreased with the EcoPlate incubation and in case of a small difference between estimated coefficients, the graph was not very representative. The clear visual distinction between the corresponding Lorenz curves was not achievable. The Gini coefficient can be a useful parameter for estimating microbial diversity with a proper choice of time point measurement. Additional computations, which involve other indexes, comparisons or correlation analysis between them, can increase the benefits of the Gini coefficient application in the EcoPlate technique.

Keywords: soil microbial communities, metabolic activity, BIOLOG EcoPlate technique, the Gini coefficient, Lorenz curve

УВОД

Метаболитната активност и структурата на микробните съобщества са важни индикатори за почвеното здраве и продуктивност. Патентованата технология на Екоплаките на БИОЛОГ предоставя метод за оценка на микробните съобщества, която се основава на използването на различни субстрати. Оптичната плътност (ОП), която се променя в гнездата по време на инкубирането на плаките в резултат на редукцията на тетразолиевото багрило се използва за изчисляването на различни индекси като параметри за микробната активност и структура. Коефициентът на Джини първоначално е бил използван в областта на икономиката за оценка на социалното благосъстояние и доходите, но намира приложение и в биологичните изследвания. Както самостоятелно, така и в комбинация с други индекси, свързани с еднородност и разнообразие на видовете коефициентът на Джини може да се използва за оценка на микробното разнообразие. В настоящото проучване е представен коефициент на Джини при различни етапи от инкубирането на Екоплаки и съответните криви на Лоренц с цел

оценка ефекта от използвания биопрепарат върху почвеното микробно разнообразие. Резултатите показват, че коефициента на Джини намалява в хода на инкубиране на Екоплаките и в случаите на малки разлики между изчислените коефициенти създадената графика не притежава диференциалност и ясна визуална разлика между съответните криви на Лоренц не се постига. Коефициентът на Джини може да бъде полезен параметър за оценка на микробното разнообразие при подходящ избор на времеви етап за изчисляването му. Допълнителни изчисления, които включват други индекси и прилагането на корелационен анализ биха могли да повишат ползите от използването на коефициента на Джини в методологията на Екоплаките.

Ключови думи: почвени микробни съобщества, метаболитна активност, техника на БИОЛОГ Екоплаки, коефициент на Джини, крива на Лоренц

INTRODUCTION

Sustainable agriculture requires the establishment and monitoring of reliable and sensitive parameters to sustain soil health and preserve soil fertility (Schloter et al., 2003, Mackay et al., 2013). Diversity, richness and functioning of microbial communities were identified as important indicators related to soil quality and they can be used for assessment of soil properties and some soil physico-chemical variables (Hermans et al., 2020). It is considered, that soil microbial diversity is important to the resilience of agroecosystems because it could affect plant productivity through processes of decomposition and mineral elements turnover (Van der Haijden et al., 2008, Maron et al., 2018). There are different approaches to the assessment of biodiversity and the structure of microbial communities (Chiarucci et al., 2011, Nkongolo and Narendrula-Kotha, 2020). Application of the BIOLOG EcoPlate technique, which is based on the microorganism's metabolic activity related to specific substrates, is another option for assessment of the structure of bacterial communities (Glimm et al., 1997). The diversity of microbial communities can be assessed by the Gini coefficient and graphically represented by the corresponding Lorenz curve. The Gini coefficient was originally employed by economists to measure income and wealth inequalities, but it was also broadly used with biological, anthropological and demographic data (Ceriani and Verme, 2011). Currently, the Gini coefficient is widely applied for the analysis of genome-expression-profiling data (O'Hagan et al., 2018, Wright Muelas et al., 2019), in population studies (Aburto et al., 2022) and in transcriptome and proteome studies (Ursu et al., 2020).

The numerical data of optical density in the wells of EcoPlates allows estimation of metabolic activity of microbial communities (average well-color development, AWCD), specific substrates` metabolic activity (substrates guilds – amino acids, amines, carboxylic acids, carbohydrates, polymers, polyphenolic compounds) and calculation of functional indexes (Ge et al., 2018). Shortly after the utilisation of the BIOLOG technique for the evaluation of microbial communities by Garland and Mills (1991) Chiarucci (1996) and Harch et al. (1997) proposed the use of the Gini coefficient for the assessment of species richness in bacterial communities. Several indexes (Shannon, Simpson, Mardalef) that assess the diversity and richness of microbial communities also can be calculated (Stefanowicz, 2006). Harch et al. (1997) considered the summarised measurements about microbial functional diversity taken at the specific incubation time on the particular BIOLOG plate as suitable for comparing different soils and as an approach subjected to less bias. According to Harch et al. (1997) the Gini coefficient (known also as Solomon`s index), Shannon index and AWCD provided similar information when used separately but analysis based on the Gini coefficient can provide additional information about the utilised carbon sources. Sharma et al. (1998) also reported the usefulness of the coefficient for the assessment of microbial diversity in soils. The Gini coefficient might also indicate disturbances in the structure of microbial species, the beneficial effects of supplements or the effect of different management strategies (Wittebolle et al., 2009).

The current study aimed to calculate the Gini coefficient based on the optical density due to the utilisation of the BIOLOG EcoPlates substrates and to compare soil microbial communities after biofertilizer application and two different doses of ammonium nitrate.

MATERIALS AND METHODS

The experiment used soil collected from the experimental field of Agricultural University-Plovdiv. The inoculated with biofertilizer soil (according to the manufacturer instruction – 1 kg per ha) and uninoculated soil were split in two and one soil sample of each treatment was fertilized with 30 or 45 mg ammonium nitrate per kg soil, respectively, which provided four variants (Table 1). After the application of biofertilizer (commercial name: Nuptak, producer - Daymsa, Spain) and ammonium nitrate all soil samples were mixed with perlite at a 3:1 volume ratio. The pots were filled with 1 kg of preliminary treated soil and each pot was sown with maize seeds of hybrid Kneja 307. The seedlings were grown for one month and after that, the rhizosphere soil was collected. Ten grams of the rhizosphere soil from each variant were placed in a flask with sterile water (90 ml), shaken for 10 min (90 rpm) and left still for another 10 min. All samples were diluted to 10^{-3} and 150 µl were used for EcoPlate inoculation. The EcoPlates were incubated for seven days at 25 ± 1 °C and the optical density was read at every 24 hours intervals on MicroStation™ Reader provided by the BIOLOG® System (Biolog Inc., USA). The optical density values obtained due to the reduction of tetrazolium dye were used for further calculations.

Table 1. Description of experimental treatments and used abbreviations

Variants abbreviation	Variants description	
	Mineral fertilizer – Ammonium nitrate, mg/kg	Biofertilizer – *Priestia megaterium*, mg/kg
30 AN	30	-
30 AN+PM	30	0.33
45 AN	45	-
45 AN+PM	45	0.33

For calculation of the Gini coefficient was used data for optical density measured at 590 nm and the 3 sets of 31 substrates in the EcoPlate were considered as replicates. The optical density of the blank well (water) was subtracted from each well and after that, the values were further subtracted from the corresponding well values obtained at the 24th-hour measurement (data normalization) to remove so-called background noise according to Urakawa et al. (2013). The negative values obtained at any step of the calculation were set to zero according to Harch et al. (1997).

The Gini coefficient was calculated according to Weiner and Solbrig (1984) and Damgaard and Weiner (2000) (Equation 1) and each final value was further multiplied by n/(n-1):

$$G = \frac{\sum_{i=1}^{n}\sum_{j=1}^{n}|x_i - x_j|}{2n^2\bar{x}} \quad \text{Eq.1}$$

where x_i and x_j are all pairs of optical density values, $\bar{x}$ – average well-color development, n – number of substrates (i.e. 31).

The Lorenz curve was created according to Lerman and Yitzaki (1984) and Ursu et al. (2020) by applying the trapezium method. Data normalization and graphs were done with MS Excel. The statistical analysis was carried out by SPSS (IBM, ver.26) with one-way analysis of variance (ANOVA) with a factor type of treatment, Tukey HSD test and level of significance $P < 0.05$.

RESULTS AND DISCUSSION

The change in the optical density (OD) in each well of EcoPlate usually progresses during the incubation but at a different pace depending on the ability of microorganisms to utilize a particular substrate. These changes in the OD are time-dependent and the obtained values affect the Gini coefficient (G) as any other indices based on the calculation of data provided by the EcoPlate. The approach towards data which are used as a source for the calculation varied significantly among authors – some used only the OD at 590 nm (Glimm et al., 1997, Grove et al., 2004, Ge et al., 2018) and others used values of both wavelengths – 590 and 750 nm (Sofo and Ricciuti, 2019).

The available literature recommendations for the calculation of average well-colour development (AWCD) – another important characteristic that can be derived from the EcoPlate technique, covered all possible measurements of 24th-hour time intervals during the plate incubation from the beginning till the 168th hour. Every recommendation about the choice of a specific time point was thoroughly justified (Cai et al., 2010, Frąc et al., 2012, Jia et al., 2013, Farkas et al., 2020). The recommendations for the Gini coefficient calculation are scarce because very few of the authors mentioned which time point they used for the estimation. Another difficulty arises from the fact that the original EcoPlate technique was based on 95 different substrates, which later were reduced to only 31 with three replications per plate. This aimed an increase of sensitivity and selectivity of the method but the calculations were done across all values of optical density provided by the wells in the EcoPlate (Insam, 1997). Németh et al. (2021) clearly stated that the calculations were based on the values taken on the 120th hour of plate incubation. Taking into account the aforementioned issues the current study presents Gini coefficients calculated on each 24th-hour interval during incubation of EcoPlate except the data taken on the 24th hour, which was used for data normalization. Such an approach provided detailed information about the effect of incubation on the calculated coefficient and it allows a less biased comparison between different variants. It can be seen (Table 2) that there was a consistent decrease in the Gini coefficient during EcoPlate incubation for all variants, and the same trend was observed by Harch et al. (1997). The distinction between the variants could be seen at any of the consecutive measurements: from the early hour – 48th till the end of the incubation – 168th hour. However, if the higher microbial diversity could be supposed for variants treated with the biofertilizer such difference could be observed only in the measurements taken on the 72nd and 96th hour. These time points correspond to the steepest part of the sigmoid curve of the average well-colour development (data not shown) and the most dynamic change of OD. Usually, the authors presented the results on particular hours such as Harch et al. (1997) – 24, 48, 72 hours or if it is a single time point it was specifically considered or was a subject of additional computations (Glimm et al., 1997). The interpretation of the Gini coefficients in relation to the microbial diversity in the samples is as follows: the higher the Gini coefficient - the lower microbial diversity and vice versa (Harch et al., 1997). In the current experiment, the variants which were treated with the biofertilizer showed low values for the Gini coefficient or higher microbial diversity at two hours – 72nd and 96th when compared to the non-treated with a biofertilizer variant (Table 2). However, the analysis of the Gini coefficients did not show significant statistical differences between experimental variants. The graphical representation of the Gini coefficient was done by the Lorenz curve. To present the difference between the Gini coefficient at the beginning of the EcoPlate incubation and at the end of the period, the variant 30 AN+PM was used (Figure 1).

Table 2. The Gini coefficient based on optical density values in the EcoPlate wells measured at 590 nm

Variants	Gini coefficient					
	Time of incubation, hour					
	48h	72h	96h	120h	144h	168h
30 AN	0.351 ± 0.072	0.215 ± 0.055	0.184 ± 0.074	0.160 ± 0.042	0.152 ± 0.037	0.145 ± 0.013
30 AN+PM	0.319 ± 0.054	0.196 ± 0.018	0.179 ± 0.010	0.173 ± 0.017	0.161 ± 0.015	0.159 ± 0.008
45 AN	0.345 ± 0.038	0.201 ± 0.016	0.179 ± 0.028	0.151 ± 0.030	0.142 ± 0.019	0.131 ± 0.016
45 AN+PM	0.378 ± 0.065	0.196 ± 0.028	0.161 ± 0.030	0.143 ± 0.012	0.135 ± 0.007	0.137 ± 0.003

The values are presented as mean ± standard deviation

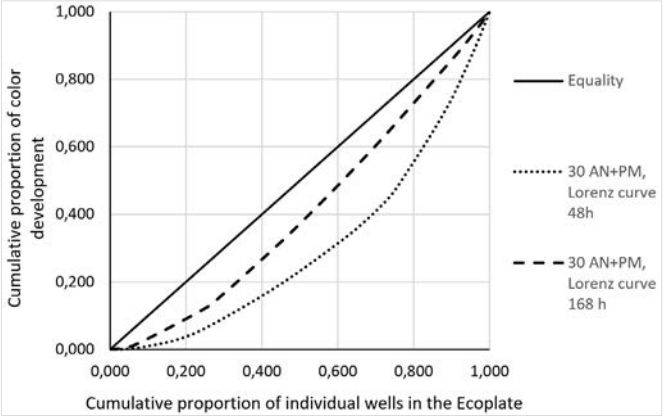

Figure 1. The Lorentz curves for microbial biodiversity of variant 30 AN+PM were calculated with the optical density data obtained at the early (48th) and at the late (168th) time point of EcoPlate incubation

The higher the Gini coefficient at the 48th hour for variant 30 AN+PM the further away from the perfect equality (black diagonal line in Figure 1) was the position of the Lorenz curve on the graph and there was a clear distinction between the Lorenz curves at 48th and 168th hour.

Weiner and Solbrig (1984) have noticed another phenomenon of the Gini coefficient and the corresponding Lorenz curves - different Lorenz curves can have the same value for G. In the current study, the created Lorenz curves which corresponded to the different Gini coefficients were almost inseparable lines on the Excel graph (Figure 2).

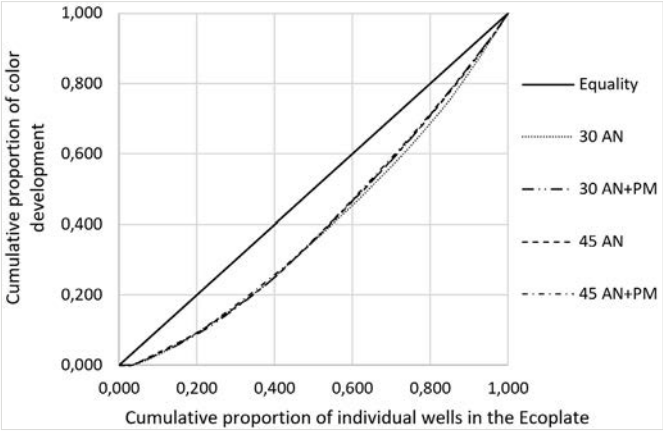

Figure 2. The Lorenz curves based on the Gini coefficients calculated on the 96th hour of the EcoPlate inoculation for all experimental variants

In this case, the comparison between different variants could be based only on the calculated Gini coefficients because the created graphs were not very representative.

In some studies, the Gini coefficient is used as an additional parameter for the estimation of microbial diversity along with other functional indexes such as the Shannon index, Shannon evenness, and Simpson index (Németh et al., 2021). However, according to Beaugrand and Edwards (2001) the Gini coefficient appears to be a better diversity estimator than any other indexes and the comparisons that can be made are reliable and satisfactory (Beaugrand and Edwards, 2001).

CONCLUSIONS

The BIOLOG EcoPlate technique is a time-sensitive method due to bacterial development and the progressing metabolic activity of soil microbial communities during the period of incubation. The changes in the optical density are due to substrate utilization and the reduction of indicator in the wells. In the current study, the Gini coefficient was calculated for the whole period of EcoPlate incubation with a starting point at the 48th hour. The resulting values for the Gini coefficients decreased with time and this trend was observed consistently for all variants. The created Lorenz curves have a clear convex slope but the attempt to present the effect of the biofertilizer at a particular time point (96th hour of incubation) graphically did not provide distinguishable lines among the experimental variants despite the difference in the calculated Gini coefficients. However, at the 96th hour of incubation, the Gini coefficients showed a higher microbial diversity for soil samples which were treated with biofertilizer. The usefulness of the Gini coefficient for the assessment of microbial diversity in soil samples could be increased by comparisons with some other indices, correlation analysis or other statistical computations.

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