

## Article

# Effect of Vineyard Floor Management on Seasonal Changes of Cultivable Fungal Diversity in the Rhizosphere

Barnabás Kovács <sup>1,\*</sup>, Csaba Dobolyi <sup>2</sup>, Flóra Sebők <sup>2</sup>, László Kocsis <sup>1</sup> and Zoltán Tóth <sup>3</sup> 

<sup>1</sup> Horticulture Department, Georgikon Faculty, Szent István University, 8360 Keszthely, Hungary; laszlo.kocsisdr@gmail.com

<sup>2</sup> Department of Environmental Protection and Safety, Faculty of Agricultural and Environmental Sciences, Szent István University, 2100 Gödöllő, Hungary; csdobolyi@gmail.com (C.D.); sebok.flora@gmail.com (F.S.)

<sup>3</sup> Department of Crop Production and Soil Science, Georgikon Faculty, Szent István University, 8360 Keszthely, Hungary; toth.zoltan@szie.hu

\* Correspondence: kovacs.barnabas.zoltan@szie.hu or kbz.georgikon@gmail.com; Tel.: +36-307209051

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**Abstract:** Vineyard floor management has been widely discussed for many decades, but it is still unclear how its intensity levels change the fungal community structure of grape rhizosphere. Our objective was to examine the density and rate of the habitats of fungi in three vineyards that differ only in the methods of tillage procedure applied, namely intensive, extensive and none (abandoned). The hypothesis was that in the cases of lower intensity or no soil tillage, there would be a higher level of fungal diversity with a lower ratio of pathogen strains in grape rhizosphere. In the course of this research, it has been determined that the level of fungal colonization of roots is the highest in the extensively managed vineyard, unrelated to season (spring and summer). Four of the five fungal genera detectable in all of the three sampled vineyards are registered as opportunist grape pathogens, however the fifth one, *Trichoderma*, is commonly used in biological plant protection. The diversity of fungal communities in grape rhizosphere, in accordance with the expectations, was the lowest in the intensively cultivated and highest in the abandoned vineyard, and it was not affected by seasons. The proportion of opportunist plant pathogen groups was higher in the intensive variant than in the other two (less-intensive variants); therefore, it is possible to conclude that soil under similar conditions but disturbed by intensive tillage methods tends to exhibit lower suppressivity.

**Keywords:** soil management; fungal community; tillage; sustainability; intensity level

## 1. Introduction

The mainstream European agriculture impact assessments (LUCAS (Land Use/Cover Area frame statistical Survey Soil) and CORINE) focus on the usability and state of European soils, in order to understand the effects of land management on sustainability and to support policy makers to design a CAP (Common agricultural policy) system based on this information [1]. The microbial community of the rhizosphere of vineyards has been subjected to a considerable amount of research [2,3] but it is still unclear how the applied soil tillage methods change the structure, composition and level of diversity of their communities [4–6].

The underground plant protection exposure of vine plantations receives much less than optimal attention both from farmers and from scientists, and the presence and the proportion of underground pathogenic organisms in the community are poorly known [4]. Some agrotechnical procedures involving mechanical soil disturbance and root cutting may pose a significant risk of penetration by providing surface for the invasion of pathogens [7,8].

About phylloxera (*Daktulosphaira vitifoliae* Fitch), which is globally the most significant pest in vineyard soil (with the exception of vineyards on immune soils) nowadays, the generally accepted assumption is that it is not the lice's chewing but the pathogenic fungi entering the plant through the damage that cause the final destruction of the plant [9]. Their composition in the rhizosphere significantly influences the extent of the damage and the rate of capital loss. Through injury on the root, fungi can enter and cause the final destruction of the vine [4]. Following this recognition, research studies have started to focus on the composition of fungal communities [10], on the ratio of pathogenic strains [11] and, primarily, on preventive procedures [12], such as soil disinfection prior to installation, use of species having more resistance with respect to their roots, deposition of dead plant parts instead of rotation and so on.

To establish a more comprehensive study of fungal diversity and to overcome the difficulties arising from the limitations of cultivation, non-cultivable dependent technologies emerged and have been widely used for about two decades. Taking into account the existing boundaries of these methods—namely the inadequacy of the detection of the actual living fungal community structure of the study sites—however, we decided to refer to cultivation-based methodology [13].

Soil tillage procedures applied on the plantations are constantly changing as new aspects need to be met, such as the changing technological environment, climatic conditions, agricultural subsidy, policy requirements and, last but not least, consumer demand, e.g., increasing demand for products produced in an environmentally conscious way [14]. The applied vineyard management methods affect the soil conditions of the plantation [15,16] and root growth [17]. This, in addition to the challenges posed by climate change, such as uneven distribution of precipitation or extreme temperature values, has to be considered by the farmer during the creation of the ideal ecological living space for the cultivated plant and edaphon alike—in other words, for farming. No-till procedures form a new trend in agriculture as, besides reduced CO<sub>2</sub> emission and fossil fuel usage, they bring about a more ecologically stable state of the soil [18,19].

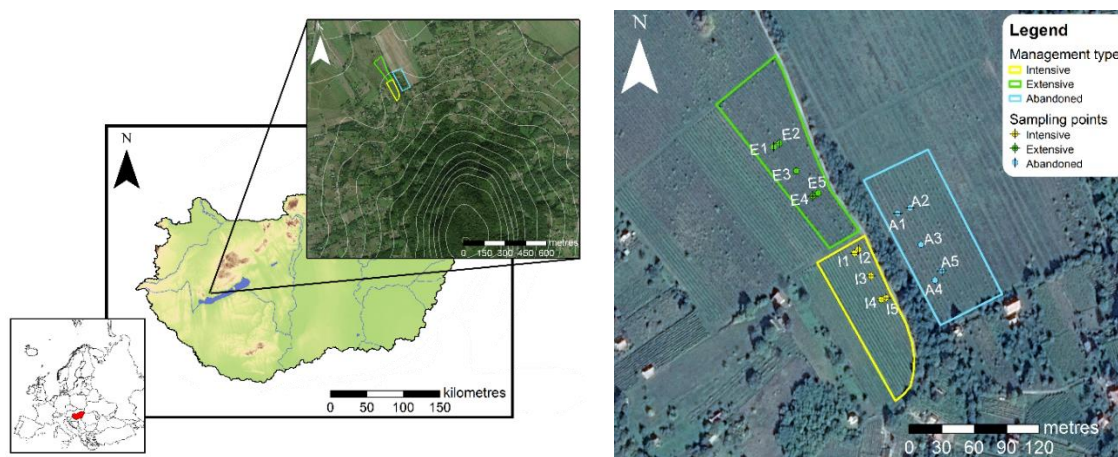
Several studies have reported that intensive tillage methods cause changes in the composition of the fungal communities of soil [4,10,18–20]. Diversity values show a decrease in intensively cultivated plantations [21,22] together with those of suppressivity. According to the reports of Huber et al. [4], Alabouvette and Steinberg [23] and Stirling et al. [24], lower levels of suppressivity appear as a side effect (often with lower organic C input) of intensive soil cultivation where the resistance of plantation soils against phytopathogenic organisms decreases.

Based on the accessed literature, the endeavor to determine whether by changing solely the intensity of the tillage methods, the level of diversity in the cultivatable fungi communities of grape rhizosphere and the proportion of the strains that can damage the crop as a pathogen will change exists. The relevance of the question is underlined by its potential benefits, since the indirect impact of reducing soil tillage intensity might result in a lesser exposure of the plant to pathogens underground, and alongside the expected increase in diversity, there would be also an ecological advantage of reducing the intensity of soil agitation.

## 2. Materials and Methods

### 2.1. Sampling

The three vineyards that were used for the examinations are located (Figure 1) within the wine region of Badacsony (Hungary), on slight slopes at the northern foot of the volcanic Saint George Hill. The tested plots are right next to each other, making them direct neighbors (Extensive (EXT): 46°85'34".4 N 17°44'19".1 E; Intensive (INT): 46°85'24.9" N 17°44'26.6" E; Abandoned (AB): 46°85'25.1" N 17°44'35.7" E). This area has a temperate, moderately cool climate (average temperature of the last 50 years 11.4 °C) with 700 mm annual precipitation, and the vineyards are not irrigated. The soil is coarse sand (VC-CV) and the texture is very loose (PD1) and non-coherent (LO) [25].



**Figure 1.** The location of the sampled vineyards (I: intensive; E: extensive; A: abandoned; 1–5: sampling points).

All three plantations were planted by the same company at the beginning of the 1980s, with grafts of Teleki 5C (Berlandieri  $\times$  Riparia) rootstock and ‘Müller Thurgau’ scion (*Vitis vinifera* L.), with a low density of plants (less than 2500 plants per hectare; originally, it was planted by 3.5 m row and 1 m plant distance (2800 plants/ha)). Ownership changed, however, and for more than 15 years, the three vineyards have belonged to different proprietors. The training methods applied on them also differ, in the intensive, mid-high cordon, in the extensive, single curtain, and in the abandoned, the last applied is Lenz-Moser cordon.

In the year of the examination, by the time of the sampling in August, exclusively disking was used for weed control between the rows, which means a disturbance within the top 20 cm of the soil, and in the rows, hoeing had been applied three times as soil tillage on the intensively managed plantation (INT). In the extensively managed vineyard (EXT), there was no procedure applied that would have disturbed the soil. Before the spring sampling, 18 months had passed with no mechanic soil tillage—which, then, was the appliance of a disk harrow 0–20 cm deep between every row. In the rows in EXT, just mowing was used as a weed control. On the third, abandoned field (AB), there had been no procedure applied in the past 15 years that would have disturbed the soil. The typical composition of natural ground vegetation in all three areas was formed by the following species: *Stellaria media* L., *Lamium amplexicaule* L., *Capsella bursa-pastoris* L., *Lolium perenne* L., *Setaria verticillata* L. and *Convolvulus arvensis* L. Due to the length of the lack of soil disturbance, *Lolium perenne* L. species showed greater abundance in the AB, and *Stellaria media* L., *Lamium amplexicaule* L. and *Convolvulus arvensis* L. lower.

Sampling was carried out during two different seasons, in spring and summer; 5–5 samples of 500 g were taken from each vineyard’s different vine rows from under the vine, from the grape rhizosphere, at a 10–20 cm and 30–40 cm depth to examine the edaphon, while 0–30 cm and 30–60 cm depth samples were taken for agrochemical examination.

Examination of the physical characteristics of the soil meant looking at soil constraint, amount of silica sand and consistency of the samples. The latter have been identified by using a penetrometer implemented in the soil surface zone (0–20 cm depth).

## 2.2. Cultivation Methods and Isolation

For the mycological examinations, ten 8–10 cm long root pieces collected from the soil samples were used, which were taken from the root system from a depth of 30–50 cm of five plants from each vineyard and were rinsed three times so there was no soil left. After cutting off the 2 mm long rootlets from the root pieces, another three rinses followed, using sterilized water to remove any outer fungi contamination. Seven rootlets per petri dish (five petri dishes per treatment; in total, 35 rootlets) were put on the surface of potato-dextrose agar (PDA from here on) with streptomycin of 10 mg/L as inoculation and—checking on them daily—were incubated for 10 days at 26 °C. Fungi-positive

inoculations and the isolations that grew out of the colonies and produced further definite cultures were registered.

### 2.3. Taxonomic Identification of the Fungi Cultivated

Non-molecular taxonomic identification of the strains was carried out on the base of their morphological characteristics. Keys of widely accepted monographies [26–29] were applied for the morphological identification of genera. For maintenance of the strains, the spores or any other propagule of the strains harvested from colony plates were suspended in 20% glycerol solution and homogenized in a tissue homogenizer tube of Potter-Elvehjem type. Suspensions were stored at  $-80^{\circ}\text{C}$  for later molecular analysis in cases where it was not possible to detect the strains at genus level according to their morphological characteristics.

For analysis of the nucleotide sequence, the internal transcribed spacer (ITS) region was used as the most currently applied region [30]. By way of morphological characteristics, the ability to form fruiting bodies, as a result of sexual process, the way of conidium ontogeny and also the conidium-forming organs and structures were examined for identification. In the course of the analysis of the ITS-region sequence, DNA was extracted from the mycelia using the MasterPure™ Yeast DNA Purification Kit (Lucigen Corporation, Middleton, WI, USA) according to the instructions of the manufacturer. The ITS region (2) was amplified by PCR using the primer pairs ITS1 and ITS4 according to White et al. (1990). PCR products were purified with a DNA, RNA and protein purification Kit (Macherey-Nagel). The purified PCR products were used in sequencing reactions using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster, CA, USA). Sequencing was performed on an ABI 3130 genetic analyzer (Applied Biosystems, Foster, CA, USA). Sequences were compared with those of all known fungal species available from the National Center for Biotechnology Information (NCBI) GenBank Sequence Database (<http://www.ncbi.nlm.nih.gov/BLAST/>).

### 2.4. Physical Parameters

Analyses of the physical parameters of the soil samples were carried out by the accredited laboratory of the Research Institute for Viticulture and Oenology (NAIK), Badacsony, Hungary. For the determination of soil texture, we applied the sieving and sedimentation (pipette method according to Food and Agriculture Organization (FAO)) method, and for the amount of silica sand, the MSZ-08-0010:1978 method, certified by the Hungarian Standards Institution, was used. For consistency, on field we used the same penetrometer (Agreto Soil Compaction Tester) on the upper level (0–30 cm) of the soil between and in the rows, with ten repetitions per treatment.

### 2.5. Calculation of Diversity Indices

The diversity of propagule communities was measured and expressed with Shannon's index ( $H'$ ) [31]. Shannon diversity quantifies uncertainty (entropy), which is calculated with the following formula by the 'vegan' package of the R statistical program:

$$H' = -\sum p_i \ln p_i$$

where  $p_i = \frac{n_i}{N}$ ;  $n_i$  = the abundance of  $i$ -th species;  $N$  = the total abundance.

The Shannon evenness index ( $J'$ ) also was determined. Although as a heterogeneity measure, the Shannon index contains evenness for a degree, the ratio of observed and maximum diversity might be used to calculate a separate evenness measure [31].

$$J' = \frac{H'}{H_{max}} = \frac{H'}{\ln S}$$

where  $S$  = number of genera in this case.

To show the relation between the species-richness indices and the evenness indices, producing values in units of ‘number of species’, in this case in ‘number of genera’, we used effective numbers, also called (Hill numbers ( $N_q$ )), as a non-parametric heterogeneity measure [32].

$$N_q = \left( \sum_i p_i^q \right)^{1/(1-q)}$$

where  $q$  = the order in which the index is dependent of rare species.

The most important Hill numbers:

$N_0 = S \rightarrow$  number of species.

$N_1 = \exp(H') \rightarrow$  exponential of Shannon index.

$N_2 = \frac{1}{D} \rightarrow$  reciprocal of Simpson index.

### 3. Results

#### 3.1. Results of Soil Analysis

According to the evaluated outcomes of accredited laboratory soil analyses, the soils differed only minimally regarding their physical and agrochemical characteristics (Table 1). There was a difference in the amount of total organic matter—in the case of INT, it was lower ( $0.5\text{--}2.4 \text{ g kg}^{-1}$ ) at both depths of sampling than in the other two plantations. Water-soluble salt content was very low (non-saline soil according to FAO standard (under EC (Electrical Conductivity) =  $2 \text{ dSm}^{-1}$  at  $25^\circ\text{C}$ ))—it was under the threshold limit ( $<0.2 \text{ g kg}^{-1}$ ) of the diagnostic method.

**Table 1.** Agrochemical and physical parameters of the composite (of five soil sampling points) soil samples.

	INT (0–30 cm)	INT (30–60 cm)	EXT (0–30 cm)	EXT (30–60 cm)	AB (0–30 cm)	AB (30–60 cm)
pH ( $\text{H}_2\text{O}$ ) *	6.9	7	6.73	6.75	6.04	6.11
pH (KCl) *	6.51	6.59	6.41	6.49	5.33	5.67
$\text{CaCO}_3$ ( $\text{g kg}^{-1}$ ) **	2.5	2.5	1.6	4.1	2.5	3.3
Total organic matter ( $\text{g kg}^{-1}$ ) **	6.6	6.6	7.1	7.6	7.5	9.0
$\text{C}_{\text{ORG}}$ ( $\text{g kg}^{-1}$ ) **	3.8	3.8	4.1	4.4	4.4	5.2

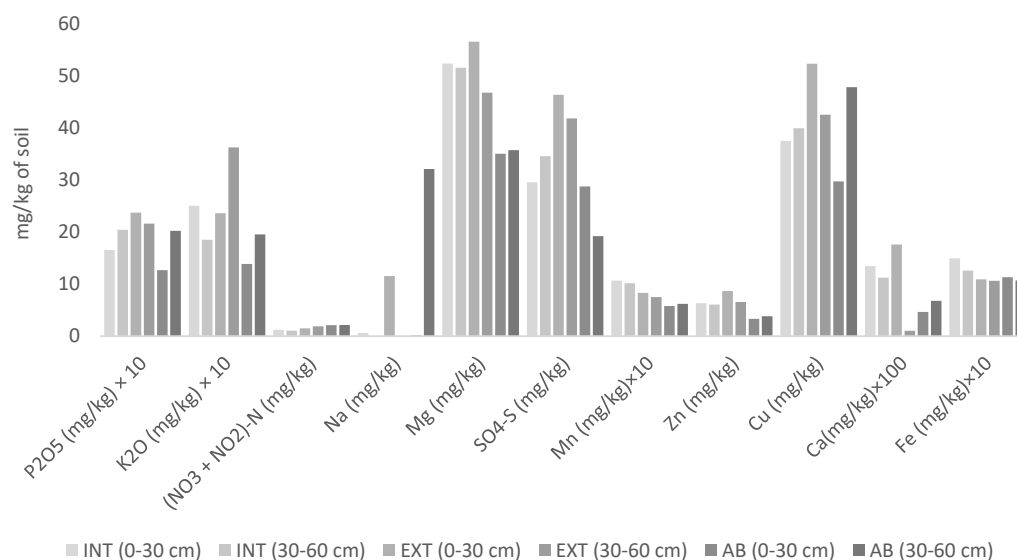
INT: Intensively cultivated; EXT: extensively cultivated; AB: abandoned. Probability of the soil test method: \* =  $\pm 0.2$  unit of value; \*\* =  $\pm 7.5\%$ .

The level of Cu (Figure 2) was higher in the samples of EXT and in the deeper samples from the AB than the ones from INT. This difference is probably due to the applied management methods, but in neither case does it reach phytotoxic levels ( $100 \text{ mg kg}^{-1}$ ) [33].

According to the criteria system of Lőrincz et al. [34], the layer of soil deeper than 30 cm is immune to phylloxera (over 85% silicate content; total ratio of humus is lower than 1%). Regarding the consistency of the soil, the intensively cultivated plantation was the least compact (still, it was compacted:  $3.9 \text{ MPa/19 mm}$ ); on the other hand, the EXT plantation exceeded it by 5% and the AB by 22.5%.

#### 3.2. Mycological Results in Connection with Vineyard Floor Management

From the grape rootlets placed on medium in the 15 petri dishes, we isolated 83 by the mycological culturing method, which represented 19 genera via their phenotypical features. Root colonization of the extensively cultivated plantation was the highest in both spring and summer, while colonization of the intensively cultivated one was the lowest. The colonization on the rootlets of all areas was higher during summer than in spring (Table 2).



**Figure 2.** Nutrient contents of soil in the sampled vineyards at two depths (0–30 and 30–60 cm) (based on the laboratory report of NAIK-SZBKI-Badacsony, an accredited soil laboratory). INT: Intensively cultivated; EXT: extensively cultivated; AB: abandoned.

**Table 2.** Ratio (%) of root colonization by fungi colonies on the sampled vineyards rhizosphere.

INT		EXT		AB	
Spring	Summer	Spring	Summer	Spring	Summer
47.8	73.1	62.5	92.4	54.8	78.9

INT: Intensively cultivated; EXT: extensively cultivated; AB: abandoned.

To examine the diversification of fungal colonies, the rootlets were incubated. In addition to registering the frequency of the types of fungal colonies forming on PDA substrate until the end of the examined period, they were also isolated. The isolations were identified by their morphological characteristics and 19 genera were defined, in the case of *Fusarium* genus, a molecular analysis was also performed in order to be able to identify the two morphologically distinct species. They were categorized in classes related to their behavior, on the bases of the following works: [4,10,11,23,35–40].

This grouping cannot be claimed as universally correct because of the different reports of species within genera and also because of the reports of alternating behavior by various studies—e.g., the *Alternaria* genus has been described as one of the most abundant plant pathogens in grape rhizosphere (even saprobes in soils) [4], while by an Italian researcher group [41], it was reported as a possible biocontrol agent against *Plasmopara viticola*, which is an another important pathogen [42], but the most possible and valid version was selected.

Five taxa (*Cylindrocarpon*, *Fusarium oxysporum*, *Fusarium solani*, *Phaeoacremonium* and *Trichoderma*) were found in all six samples and *Pythium* was present in samples collected in both seasons of EXT and AB (Table 3).

In all three vineyard management methods, *Fusarium* proved to be the most common genus and it was possible to distinguish two morphotypes (two species) based on colony and microscopic morphological features. *Fusarium oxysporum* had a high abundance in the INT plantation (30.0–50.0% of all fungal propagates in the sample) increasing with the approach of summer, with *Fusarium solani* showing a higher frequency in the samples collected at spring, though it has the same seasonality.

**Table 3.** Percentage (%) of frequency of fungal morphotypes isolated from the surface of vine-roots, grouped by the examined vineyard management methods and seasons.

Morphotype	Classes of Behavior	INT		EXT		AB	
		Spring	Summer	Spring	Summer	Spring	Summer
<i>Acremonium</i> sp.	Antagonistic	0	0	1.63	0	3.76	2.03
<i>Alternaria</i> sp.	Pathogenic	0	0	8.94	2.42	2.26	0
<i>Aspergillus</i> sp.	Pathogenic	0	0	1.63	1.61	2.26	0
<i>Coniothyrium</i> sp.	Antagonistic	0	3.08	0	0	0	0
<i>Cylindrocarpon</i> sp.	Pathogenic	34.06	13.08	27.64	12.90	27.82	13.51
<i>Doratomyces</i> sp.	Saprophytic	0	0	0	0	1.50	0
<i>Fusarium</i> spp.		47.83	59.23	36.59	35.48	30.83	28.38
<i>F. oxysporum</i>	Pathogenic	30.43	50	13.01	18.55	10.53	15.54
<i>F. solani</i>		17.39	9.23	23.58	16.94	20.30	12.84
<i>Glomastix</i> sp.	Antagonistic	0	0	1.63	4.03	2.26	3.38
<i>Myrothecium</i> sp.	Antagonistic	0	0	0	4.03	1.50	0
<i>Mortierella</i> sp.	Saprophytic	0	0	1.63	1.61	0	2.70
<i>Mucor</i> sp.	Saprophytic	0	0	0	2.42	0	2.03
<i>Oidiodendron</i> sp.	Antagonistic	0	0	0	0	2.26	2.70
<i>Paecilomyces</i> sp.	Antagonistic	0	0	1.63	0	3.01	0
<i>Penicillium</i> sp.	Saprophytic	0	0	1.63	4.03	0	3.38
<i>Phaeoacremonium</i> sp.	Pathogenic	10.87	13.08	5.69	10.48	11.28	17.57
<i>Pythium</i> sp.	Pathogenic	0	0	4.07	8.87	3.01	12.84
<i>Torula</i> sp.	Saprophytic	0	0	0	0	2.26	0
<i>Trichoderma</i> sp.	Antagonistic	7.25	11.54	7.32	9.68	5.26	7.43
<i>Verticillium</i> sp.	Saprophytic	0	0	0	2.42	0.75	4.05
TOTAL		100	100	100	100	100	100

INT: intensively cultivated; EXT: extensively cultivated; AB: abandoned.

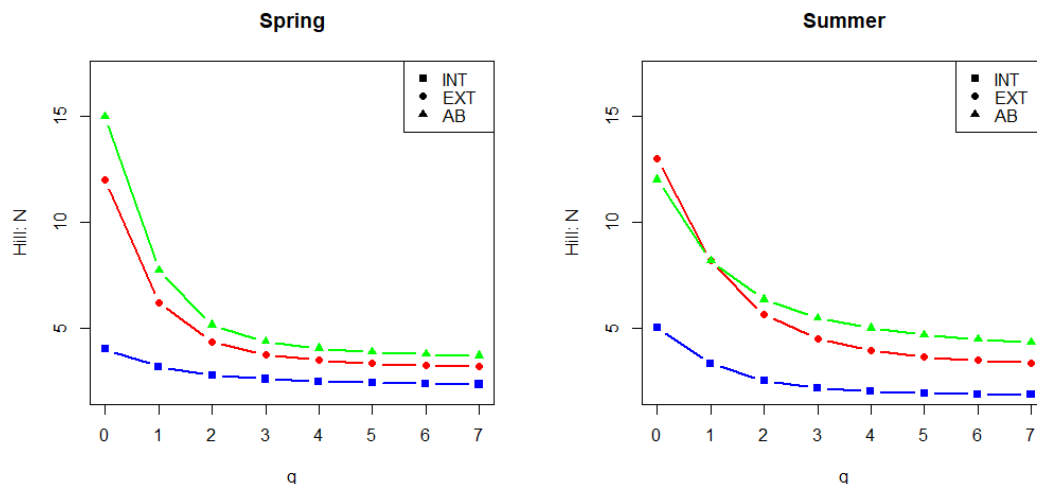
Regarding the morphotype richness of fungal colonies (expressed by the number of morphotypes) and the ratio of morphotype diversity, the values of the intensively cultivated plantation were noticeably lower than in the other two cases (Table 4).

**Table 4.** Morphotypes, colonies and Shannon diversity indexes of the examined vineyard management methods and seasons.

Index	INT		EXT		AB	
	Spring	Summer	Spring	Summer	Spring	Summer
Number of morphotypes	4	5	12	13	15	12
Number of colonies	138	130	123	124	133	148
Shannon diversity (H')	1.15	1.2	1.83	2.10	2.04	2.10
Shannon evenness (J')	0.83	0.74	0.73	0.82	0.75	0.85

INT: Intensively cultivated; EXT: extensively cultivated; AB: abandoned.

According to the effective numbers, the AB plantation has a slightly higher diversity than the EXT one at spring, and both had higher levels than the INT, but in summer, it is not distinguishable (Figure 3).



**Figure 3.** Hill diversity ( $N_q$ ) of differently cultivated vineyards at spring (left) and summer (right). INT: Intensively cultivated; EXT: extensively cultivated; AB: abandoned.

#### 4. Discussion

The EXT vineyard is managed by certificated organic methods, which means only copper (Cu)-based plant protection might be applied against the most damaging above-ground fungus, *Plasmopara viticola*, the causal agent of downy mildew. Furthermore, in the case of AB, a decade ago in Hungary, copper was the most often used pesticide, and in the last non-cultivated period, it leached to deeper zones. However, none of these quantities are high if we compare them to the report of the copper level of the soils of European vineyards [43], although its presence has a possible impact on fungal [44] and bacterial communities [33]. In soils, fungi are more sensitive than bacteria or archaea [45] to Cu pollution, which could cause a negative effect on the fungal communities on the EXT plantation (0–30 depth) or at the AB treatment (30–60 cm depth) but it does not occur at the diversity level [46,47]. However, soil pH and disturbance have a greater effect on bacterial than fungal communities [48] and they also probably played a role in the higher diversity level of fungi with the AB treatment compared to the other two [33].

According to the evidence of the listed source articles, six of the nineteen detected genera have been registered with pathogenic behavior. These are *Acremonium*, *Aspergillus*, *Cylindrocarpon*, *Fusarium* (both *F. oxysporum* and *F. solani*), *Phaeoacremonium* and *Pythium*. Three of these (*Cylindrocarpon*, *Fusarium* and *Phaeoacremonium*) were present in all six samples and *Pythium* was found in the EXT and AB vineyards in both seasons.

In grape rhizosphere, Granett et al. [10] identified *Fusarium* sp. and *Pythium* as frequent pathogenic fungi which were also detected. They listed the *Trichoderma* strain, which was found in all the six samples, as especially rare.

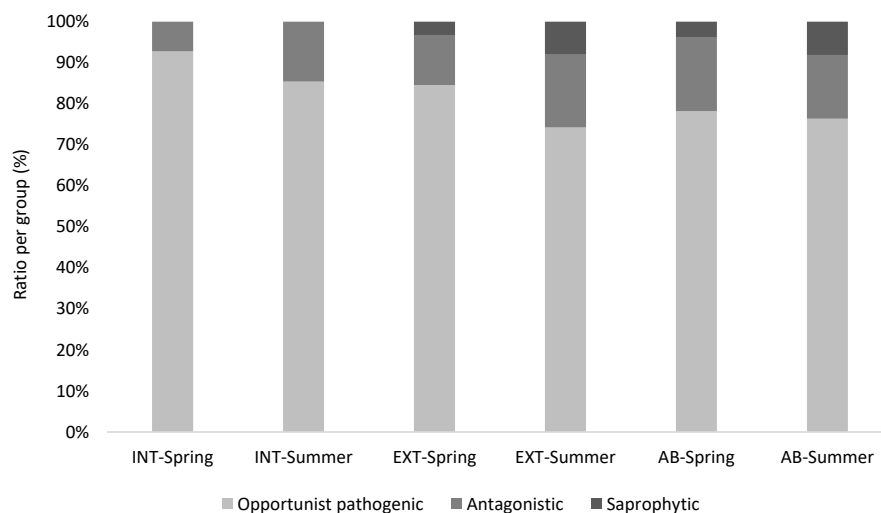
In their studies about the final destruction of vine caused by the connection between the pathogenic fungi and phylloxera, they found a seasonal fluctuation in the number of lice, which was inversely proportional to the level of damage. With these results, they established a hypothesis that fungi are less active in summer because of higher temperatures, but this was denied by Omer et al. [35]. The experiment they conducted with *Fusarium oxysporum* strains in vitro showed that sporulation, growth and infectivity increased with temperature. These experiences with in situ conditions confirmed this as in summer, there was a higher number of this strain than in samples taken in spring.

*Cylindrocarpon*—just as with *Phaeoacremonium*—is responsible for one of the most serious damages in vineyards globally, wood disease parasitic complex [44,49]. It has been found that these strains show a similar seasonal fluctuation as *Fusarium solani* but in even higher abundance.

*Cylindrocarpon* and *Pythium* showed a contrary seasonal fluctuation (lower abundance in late summer and higher in spring samples) in grape nursery soils for Collier et al. [50].

In accordance with the expectations, the diversity of fungal communities was 59–75% lower at the INT vineyard than in the EXT or AB vineyards per season (Table 4). This difference confirms data recorded by Varanda et al. [51] and confirms the findings of Winter et al. [52]; however, these results are in contrast to findings of Hagn et al. [53].

The proportion of the opportunist plant pathogen groups (Figure 4) was higher (*Spring*: +8.20 *Summer*: +11.18%) in the INT vineyard compared to EXT and AB (+14.54 and +9.03%, respectively) in line with the expectations based on the work of Hernandez and Menéndez [6].



**Figure 4.** Ratio per group of behavior (%) based on the data used for Table 2. INT: Intensively cultivated; EXT: extensively cultivated; AB: abandoned, in two seasons of 2015, spring and summer.

Examining seasonal changes within diversity values, it was found that in contrast with the ratios in INT (7.37%) or EXT (10.35%), in AB, there were almost no seasonal changes (1.86%), which might be due to the more compact and undisturbed soil qualities as climatic effects had less impact on fungal communities, in accordance with the lack of physical and chemical changes in the soil.

## 5. Conclusions

Comparing soil cultivation methods used in vineyards in both economic and ecological aspects is important for vine growers. Finding solutions that can improve the combination of multiple factors is particularly important when the effects of climate change put agronomists into the difficult position of having to face many challenges and constraints at the same time.

Soil used by agriculture is one of the most important natural resources, so maintaining its quality and quantity is an important task. If farmers can create a favorable state for cultivated crops while keeping ecological values, it is possible to speak of a truly sustainable system.

The indicators of consistently higher diversity values measured in a longer period are indicators of the ecological stability of the soil. The inversely proportionate changes of the diversity parameters observed in the different vineyard management methods and the opportunistic pathogenic strains may lead us to form some conclusions about the estimated suppressivity.

According to the findings concerning the ratio of opportunist plant pathogen fungi, it has been concluded that along abiotic and biotic parameters, vineyard soil that is disturbed by intensive tillage methods has a lower level of suppressivity than the less cultivated, more ecologically balanced one. This ecological balance is clearly illustrated by the low ratio of seasonal change in the high diversity shown in the abandoned area. Furthermore, the diversity of the fungal community decreased in the case of more intensive tillage, while in the case of AB and EXT, there was no significant difference. Although it was not possible to perform statistical analysis because of the nature of these data, the expected low diversity of INT has been confirmed by both the Shannon index ( $H'$ ) and Hill numbers.

By being able to reduce the degree of soil disturbance and, at the same time, to provide a more favorable environment for soil flora and fauna, which perform mineralization, degradation, plant protection exposure or reduction, it is possible to achieve a higher ecosystem service capacity in the given agro-ecosystem. This may be supplemented by the advantage that associations can be formed on the less-disturbed soil surface, which increases the diversity of the vineyard and helps the farmer's work above the surface.

In finding a more adequate balance of intensity in vineyard management methods—which depends on many factors and presupposes multilevel responses—farmers would be able to change their practices to more sustainable ones, preparing themselves for meeting new pathological and environmental challenges.

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