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ORIGINAL PAPER

Litterbag-NIRS to Forecast Yield: a Horticultural Case with Biofertilizer Effectors

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Abstract

The Litterbag-NIRS method demonstrate and study the microbial biodiversity of soil in an indirect way, observing the quality decay of ground hay, under real field conditions. Three experiments pertaining to a complex microbial consortium inoculated into six horticultural species used litterbags buried for 60 days. The litter was examined, by an SCiO™ smart-NIR spectrometer, to extract information on the type of transformation that had taken place. Chemometric analyses of single spectra were conducted to compare any variability in three experiments. The partial least squares method was used and cross-validated to associate the observed equivalent yield indexes (YI) to the NIR spectra averaged over each productive plot, in each trial, as well as in the pooled dataset. The cross-validated R^2 values of the three experiments ranged around 0.66, and the inaccuracy of the estimates fluctuated at around ± 5%. The pooled calibration ($R^2 = 0.55$) showed the presence of outlier treatments, and a marked spectral correlation ($R^2 = 0.77$) with the 1031 and 986 nm wavelengths. In parallel, a complex of 22 NIRS-predicted variables related to chemical decay of the hay-litter, soil characteristics, and soil microbiology was obtained and partially associated to the YI and to microbial inoculation effects. The Hay-Litterbag-NIRS method can be considered useful to indirectly demonstrate that microbial fertility is an integral part of soil fertility, as evidenced by the significant correlations and predictions of the crop yields, and by the unraveling of the tangle of plant-soil relationships.

Keywords Hay litterbag · NIRS · Horticultural products · Predictive yield · Biofertilizers · Soil microbial activity · Fingerprinting

1 Introduction

The soil-food web lies at the core of all soil ecology. In fact, plants are not simply immobile objects that absorb nutrients and water wherever their roots happen to go. A total of 5–21% of the photosythate (organic C) of each plant is used to produce root exudates and root turnover (Balestrini et al. 2015) These carbon molecules feed the microbiota located in the area closest to the roots which Lorenz Hiltner defined in 1904 as the rhizosphere (Hartman et al. 2008). Therefore, the soil microbiome around the roots of any plant is controlled by the surrounding soil type and the kind of root exudates, which change according to the species and variety of the plant (Berg and Smalla 2009). Root exudates may also change during the growth phase of a plant or in concomitance with plant pathogens and infections, when the immune system of the plant is activated (Finkel et al. 2017). A wide variety of plant species produces a wide variety of exudates and enhances a livelier, healthier, and more competitive soil food web. Different plants attract different cross-sections of the bacteria and fungi in a soil, initially according to the composition of the unique root exudates of each plant (Glick and Gamalero 2021). The soil-root interchange also affects the health of a plant, as pathogenic microbes need to fight more to proliferate in the rhizosphere. All the microorganisms that are present and the larger organisms that feed on them function like natural fertilizers. They store organic compounds in their bodies, which are recycled once they die and are made available to the plants in the soil. The soil-food web is essential for the nutrient cycles that take place and can be used to predict the functioning of these cycles

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and the correlated ecosystem services (de Vries et al. 2013). In addition, the population and activity of a microorganism are closely correlated with the soil structure, as fungi grow hyphae and form a large network, while bacteria secrete slimes that become attached to other particles and, in the same way, create aggregations of soil particles. Nematodes, protozoa, and various arthropods move around the soil in search of food, thereby loosening the soil and aerating it. One way of measuring the biological quality of soil is to resort to the QBS-ar (Soil Biological Quality Index) created by Parisi et al. (2005), which is based on the ability of micro-arthropods to proliferate in the soil. The presence of microorganisms and the soil food web are indispensable for all ecosystem services.

However, many soils around the world have become impoverished as a result of poor agricultural practices. Moreover, an ever-increasing part of intensive agriculture is now carried out in soilless systems, which are based on the use of chemicals and have no need of microbes in soil; on the contrary, they try to reduce it to a condition of pseudo-sterility.

The chemical aspect prevails in the concept of soil fertility. The presence and effects of the microbial populations that swarm about in the brown world are ignored to a great extent. The only studied and feared aspects are the negative ones, that is, root pathogens and soil fatigue.

FAO (2006) defined biofertilizers as "products containing living or dormant micro-organisms, such as bacteria, fungi, actinomycetes and algae alone or in combination, which on application help to fix atmospheric N or solubilize/mobilize soil nutrients in addition to secreting growth-promoting substances."

Nowadays, positive microbes, such as arbuscular mycorrhizal fungi and plant growth-promoting bacteria, which are related to beneficial agronomic contributions, are beginning to appear in almost all advanced agriculture models (Aguilar-Paredes et al. 2020; Schütz et al. 2017), but mainly in those labeled as greening ones, and such microbes are looming on the horizon of human health. Their pathway is steeply uphill, and the goal is still distant, because the results are sometimes variable and uncorrelated when transferred from laboratories to real soils. This is even more reason why, in less intensive production systems, such "good" microbes should be a flag of the soil fertility mechanisms at play in the restoration process and the green carbon sink. However, the practical knowledge of the real microbial biodiversity of cultivated soils is still far from clear. Agronomic sciences deal with this complexity with means that are generally too sophisticated and expensive for economic and transferable practice (Daniel 2005). The soil biota in precision farming operations is beyond any possible application, for now. The problem with soil microbes is that they are invisible, but above all, there are numerous and completely different from each other. Therefore, if the long route to obtaining knowledge about them is closed, is there any hope of a shortcut? The answer is yes. Quick ways of indirectly evaluating the microbe functionality of soil do in fact exist. The first published shortcut refers to the Litterbag-NIRS method (Masoero et al. 2018). Using this method, the degradation of a standard forage, ground to 3 mm, buried in cultivated soil at a depth of 10 cm for 60 days, was observed by scanning its NIR spectra without the need to prepare a sample, and without the need to weigh the samples: only spectra were required. A smart NIRS device (Goldring and Sharon 2016) allows a quick analysis to be made, provided that a limited number of replicates are foreseen. The simple random forest algorithm included in the software device can define the average classification of different groups of spectra in a simple comparative model (group fingerprinting of, i.e., conventional vs. organic; C-control vs. M-microbially inoculated).

In the present work, the aim has been to ascertain whether a spectral correlation between litter degradation in litterbags and the yield of some horticultural crops is positive and, in such a case, to what extent the relationship can be predicted 60 days after the belowground decay of the litterbag. Moreover, the challenge has been to graduate the links between the litterbag-NIRS variables and the whole yield, considering all the putative microbial sources—between and within theses—as influencers of the growth-promoting substances.

2 Materials and Methods

2.1 Crop Experiments

Three asynchronous models were utilized to compare the production of horticultural crops under a normal conventional control (C) and under a biofertilized model improved with a microbial consortium containing arbuscular mycorrhizal fungi (M). Three experiments were conducted and analyzed separately, and the results were then pooled together. The collection of data and spectra from three experiments is shown in Table 1. The first two experiments were conducted in a light greenhouse in the UNISG (Pollenzo, Italy) productive garden in the fall of 2018 and in the spring–summer of 2019. The third experiment was conducted in vegetable garden boxes at SERMIG (Torino, Italy), in the spring–summer of 2020. The main aim of the experiments was to evaluate the results of the application of a microbial consortium (Micosat F®, by CCS – Aosta) (Baldi et al. 2020, 2021) to certain horticultural crops. Before transplanting, half of the plantlets were inoculated in a bath with a biofertilizer solution, and the others were inserted into pure water. In the last trial, a reinforcement inoculation was used in a third theses (M+). In order to scale and weigh the different specific yields, the results of the replicate plots were

Table.1 Plan of the trials over the years and the centers

Year	Center	Code	Crop	Akronym	Species	Cultivar	No. plants
2018	UNISG	U18	Lettuce	Lac	*Lactuca sativa*	-	72
2018	UNISG	U18	Radish	Rad	*Raphanus sativus*	-	164
2019	UNISG	U18	Tomato	CDB	*Solanum lycopersicum*	*Cuore di Bue* GIGAWACK®	18
2019	UNISG	U19	Tomato	Cos	*Solanum lycopersicum*	*Costoluto*	18
2019	UNISG	U19	Bell Pepper	Pep	*Capsicum annuum*	*Quadrato di Carmagnola*	72
2020	SERMIG	U19	Endive	Sca	*Cichorium endivia*	Scarola	72

averaged over 100 for each crop\cultivar, and an equivalent yield index was then calculated.

A second aim—which has become the first in the present work—was to evaluate the efficiency and utility of the Litterbag-NIRS method to fingerprint the use of the biofertilizer and to ascertain the feasibility of the method for the prediction of yields.

2.2 Litterbag Decay in the Medium Term

When a litterbag is unearthed, its components are the same as those of the initial hay, albeit modified by the microbiological action of the microflora present in the hay itself (not sterilized) but above all by the surrounding microflora of the soil that flows into the litterbag matrix as the result of the movement of water, roots, fungal filaments, microarthropods, etc. Figure 1, which was obtained from a Doctoral Thesis (Santoni 2015), highlights the average decay, at day 60, of litter compounds issued from three forage sources in two experimental sites: although all the components decreased in weight from the time of burial, it can be observed that the relative % of some compounds increased in incidence over time, and can be considered as slow or constant: ash (+141%), hemicellulose (+74%), lipids (+72%), NDF digestibility (+54%), crude proteins (+18%), digestible NDF (+17%), and ADL (+8%). On the other hand, some other compounds were reduced in percentage, and can be considered "rapid": indigestible NDF (−56%), crude fiber (−53%), cellulose (−44%), ADF (−30%), NDF (−23%), and gross energy (−9%). The average deviations observed during the growth of twelve green crops, examined at four stages (Tassone et al. 2014), are plotted in Fig. 1. Interestingly, the allometry in the green crops appears symmetrically balanced with the litterbag decay, with a parabolic R^2

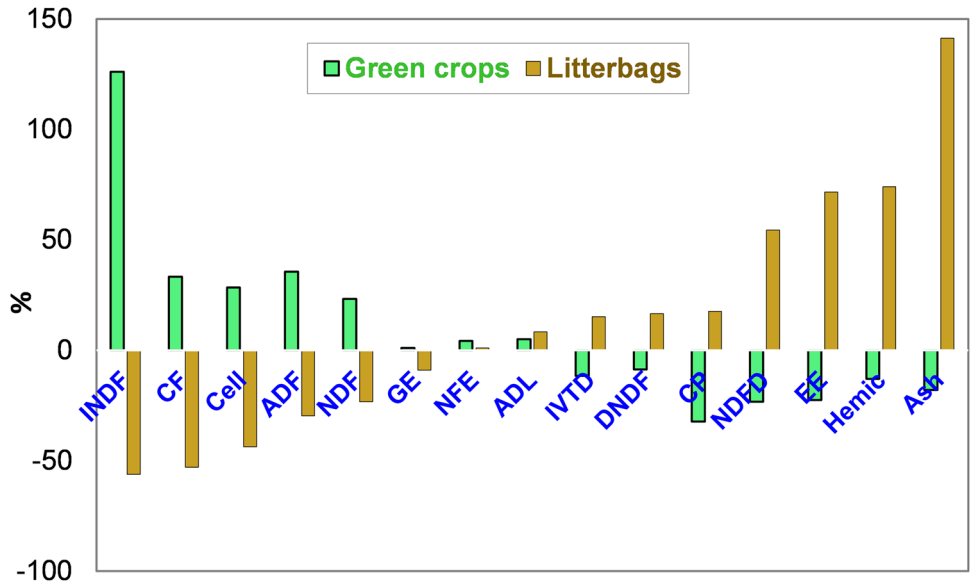

Fig. 1 Comparison of the allometry of compounds (as average % deviation during the growing time) in twelve green-crops (Tassone et al., 2014) and of the 60-d % decay in litterbags from three sources (Santoni, 2016). *INDF* indigestible NDF (126,−56), *CF* crude fiber (33,−53), *Cell* cellulose (28,−44), *ADF* acid detergent fiber(35,−30), *NDF* neutral detergent fiber (23,−23), *GE* gross energy (1,−9), *NFE* nitrogen-free extracts (4,1), *ADL* acid detergent lignin (5,8), *IVTD* in vitro total digestibility for ruminants (−12,15), *DNDF* digestible NDF (−9,17), *CP* crude protein (−32,18), *NDFD* NDF digestibility (−23,54), *EE* ether extract (−22,72), *Hemic* hemicellulose (−13,74), Ash (−18,141)

of 0.47 (Fig. 2). This simply indicates that what has been constructed early on will later be destroyed underground, and vice versa.

2.3 Hay Litterbags and NIR Spectra Upload

The basic vegetable matrix used for the preparation of the litterbags was hay for small animals ("Vita Verde Small Animal Alpine Hay," by Vitakraft pet care GmbH & Co. KG, Bremen, Germany) (Table 5). The probe was prepared after grinding the hay to 3 mm using a Retsch SM 100 cutting mill. About 3 g of hay was packed into 5 × 10 cm (1.5 mm mesh) single layer polypropylene net rectangles, which were then sealed with a few staples. A plastic label was applied for identification purposes and to facilitate retrieval. The litterbags were placed underground at a depth of 8–10 cm along a row of cultivated plants, at a distance from drips. They were extracted and transferred to the laboratory after about 40 days, dried in an oven at 45 °C, gently cleaned, and stored at room temperature until NIRS examination. The brushed probes were opened, and the surfaces of both sides were examined, by means of a miniature NIRS spectrophotometer connected wirelessly to the Internet (SCiO™ v. 1.2, Consumer Physics, Tel Aviv, Israel; Goldring and Sharon 2016), using a magnetic spacer capsule, measuring 9 * 40 mm. The SCiO™ instrument operates by illuminating the object that has to be analyzed with a blue LED light flash. The reflected signal is detected by sensors over the 740–1070 nm NIR range (1 nm interval), immediately verified for quality and then sent to the SCiO center. The scan\verification\registration operation takes 4 s and works best if the internet connection is fast (> 20 Mb s^{-1} in uploading). Each sample in the collection was registered unequivocally via alphanumeric identification attributes. Two scans were made on each side of the residual litterbag. The NIR spectra were progressively uploaded, via internet, onto the SCiO™ repository collection.

2.4 Hay Litterbag-NIRS Variables

Thanks to the use of decoding equations presented in a previous work (Baldi et al. 2020), the Litterbag dataset was provided with a complex of 22 Litterbag-NIRS (LBN) variables derived from the NIR spectra (ref. Table 5), of which 16 were related to the chemical composition of the hay, 2 to the characteristics of the soil (NO_3^--N and NH_4^+-N), and 4 to the soil microbiology, respiratory capacity, types of cd microbial populations, rapid (r-strategists), and slow (k-strategists), and their differences (r-k). An indirect evaluation system of the types present in the active microflora of the soil consists in presuming that the microbial populations of the so-called rapid populations (r-strategists) are responsible for the rapid disappearance of any less resistant components present in the natural litter, while the so-called populations of the k-strategists are the ones that modify the least degradable substances and characteristics more slowly (Fontaine et al. 2003; Blagodatskaya et al. 2009). Considering the composition of the starting hay, the rapid microbes can be considered proportional to the average % decrease in the rapid compounds, while the k-microbes can be considered proportional to the average % of the resistant compounds, that is, those that increase over time. The difference between the r- and k-strategists may be an important index that can be used to represent the relative dynamics between the two kinds of litter compounds.

2.5 Statistical Analysis

When operating on a computer and using TheLabScio™ software, it is possible to manage the front-end of the database, add variables, and to export and import added values,

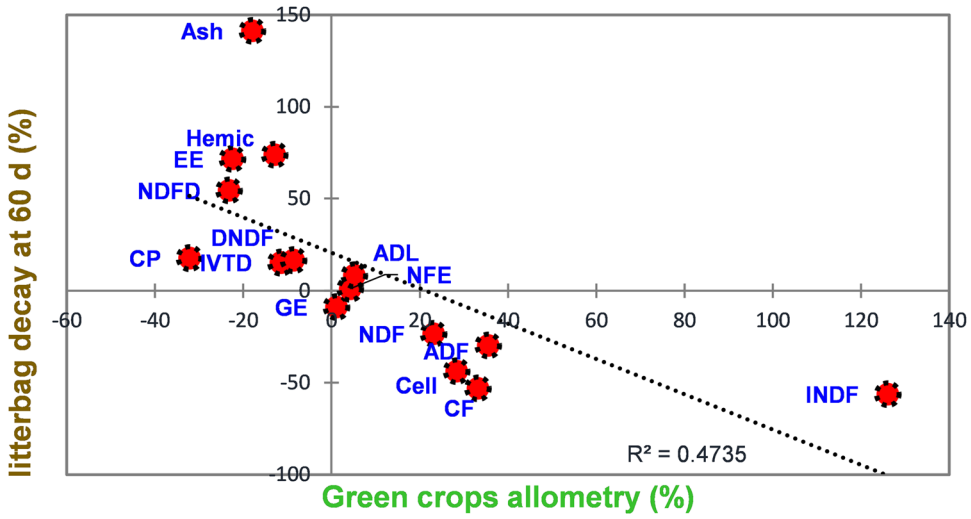

Fig. 2 Strong negative correlation of the compounds in the biomass allometry of twelve green-crops (Tassone et al., 2014) paired to the 60-day decay in the litterbags from three herbs sources (Santoni, 2016). *Hemic* hemicellulose, *EE* ether extract, *NDFD* NDF digestibility, *DNDF* digestible NDF, *CP* crude protein, *IVTD* in vitro total digestibility for ruminants, *ADL* acid detergent lignin, *NFE* nitrogen-free extracts, *GE* gross energy, *NDF* neutral detergent fiber, *ADF* acid detergent fiber, *Cell* cellulose, *CF* crude fiber, *INDF* indigestible NDF

on which qualitative fingerprinting algorithms, based on random forest classifiers for qualitative variables, can be built. The SCiO algorithm was applied to a single spectrum dataset, with appropriate filters, to fingerprint the effects of the crop\cultivar and of the microbial inoculation.

In our case, most of the work was carried out on spectra downloaded in csv format and then imported in a format compatible with the WinISI II v1.04 software (FOSS NIRSystem/Tecator, Infrasoft International, LLC) for chemometric processing, by means of the partial least squares (PLS) method, and systematic cross-validation. The models applied to the normalized and first derivative reflectance spectra were defined in a previous work (Baldi et al. 2020) and resulted in 22 LBN-predicted variables. Moreover, the spectra of the subgroups were averaged and then fitted to the yield index, as already done in the work of Cugnetto et al. (2021), although some outliers were identified beyond the $t < 2$ limit and were consequently excluded.

Further statistical analyses of the LBN variables were also conducted on EXCEL spreadsheets, expanded with the XLSTAT statistical software (Addinson, 2021), using one-way ANOVA and a Levene test on the control and microbial classes, and PLS models, to disentangle the most meaningful relationships among the yield values and the LBN variables. The graphs built in EXCEL were exported in high-quality 700 dpi format, thanks to the use of the XL Toolbox NG (Kraus 2014).

3 Results

3.1 Inoculation Treatments and Fingerprinting of the NIR Spectra

The microbial inoculation treatment was only successful in the SERMIG trial (Table 2). In the other two trials, the yield index was on average negative, and significantly so in the tomato CDB plants. The six crops influenced the features of the litterbags, with an average correct classification of over 82% (vs. the threshold of 17%) in both the control and in the microbial litterbags, with the exception of the tomato cultivars (Table 3). As far as the microbial effects are concerned, a significant fingerprinting model was overall obtained for both the control (65%) and microbial treatments (73%), but it was not valid for the tomatoes or bell pepper. Interesting, the fingerprinting of the CC and MM litterbags resulted to be inversely and directly associated with the effect size of the inoculation, and a bilinear regression (Fig. 3) thus reached a significant fitting (0.78).

3.2 Calibration and Validation of the Yield Index, Microbial Treatment, and Trial Effects

It was possible to obtain a significant estimate of the equivalent yield indexes of the studied theses from the 343 NIR spectra of the litterbags, averaged over 63 measured parcels that were then merged in 13 theses from the 6 horticultural crops (lettuce, radishes, two tomatoes, bell peppers, escarole) (Table 3). The cross-validated R^2 value was on average equal to 0.66 for the three trials, with an inaccuracy of the estimate fluctuating around $\pm 5\%$. Some outliers were observed in each trial (Figs. 4, 5, and 6). It should be noted that the somewhat optimistic R^2 values represent the calibration mode, but the cross-validation shows a significant relationship between the averaged NIR spectra and the plot yield equivalent (Table 4). When the averaged spectra of the theses were merged (Fig. 7), a less reliable pattern was observed. In fact, the cross-validated R^2 values descended to 0.55, and three outliers were observed. When analyzing the linear regression spectra, in the search for wavelengths, two special points were identified at 1031 and 986 nm, and the R^2 rose to 0.77. The evaluation of the microbial treatment, on the pooled and P19 models, for tomatoes and pepper, was not reliable, while R^2 rose to 0.47 and 0.80 in the other trials.

Table.2 Effectives of the litterbag spectra and parcels for the six crops from the two centers, and differences in yield index between the control and microbial consortium inoculated crops

Yield index										
No. spectra	No. parcels	Code	Crop	Akronym	C Control	M Microbial	M+ Microbial+	SD	% dYI[1]	*p*
88	12	U18	Lettuce	Lac	102.6	97.4		26.2	−5	*0.749*
82	12	U18	Radish	Rad	102.5	97.5		25.5	−5	0.756
25	7	U19	Tomato *Cuore di Bue*	CDB	103.3	96.7		4.1	−6	0.050
21	5	U19	Tomato *Costoluto*	Cos	108.6	93.5		20.0	−14	*0.130*
61	9	U19	Bell Pepper	Pep	105.3	94.7		29.2	−10	*0.510*
66	18	S20	Scarola	Sca	93.2	103.3	104.3	10.0	+11	*0.022*
343	63	Means			102.6	98.2				

SD standard deviation, *dYI* effect size of the microbial treatment = % deviation of M from C = $M*C^{-1}–1*100$

Table.3 Correct classification % based on the litterbag NIR spectra of the crops within year[1], over years[2] in the control, and microbial treatment[3], of the control (CC%) and microbial (MM%) treatments, and deviation of M/C in the yield index (dYI) with Pearson correlations

Crop	Ak	Year	Year	C-Control		M-Microbial		CC%	MM%	% dYI
			% Correct crops[1]	No	% Correct crops[2]	No	% Correct crops[2]	% Correct treatment[3]	% Correct treatment[3]	
Lettuce	Let	18	0.94 *	44	83 *	44	86 *	72 *	93 *	−5
Radish	Rad	18		42	82 *	40	90 *	85 *	82 *	−5
Tomato *Cuore di Bue*	CDB	19	0.98 *	12	83 *	13	20	63	69	−6
Tomato *Costoluto*	Cost	19		11	66 *	10	50 *	75	70	−14
Bell Pepper	Pep	19		32	84 *	29	93 *	73 *	62	−10
Scarola	Sca	20	0.88 *	23	84 *	43	96 *	50	90 *	+11
Total				164		179		65 *	73%*	
			r (CC%, MM%; dYI)					−0.72	0.67	

CC% % of correct classification of the control in microbial type, *MM%* % of correct classification of the microbial in microbial type

[1]Threshold 1/3

[2]Threshold 1/6

[3]Threshold ½, *dYI* % deviation of M from $C = M*C^{-1}-1*100$

*% correct classification significant at $p \leq 0.05$

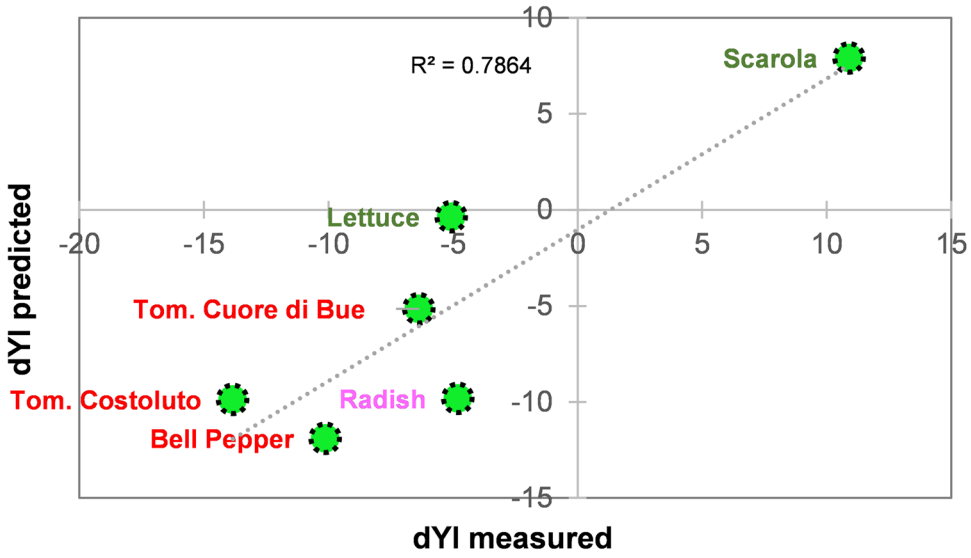

Fig. 3 Bilinear regression of the microbial effect size (dYI (M/C-1)*100) on the fingerprinting of the control (CC%) and microbial (MM%) litterbags: scatterplot of the predicted/measured values for the six crops with % in axes

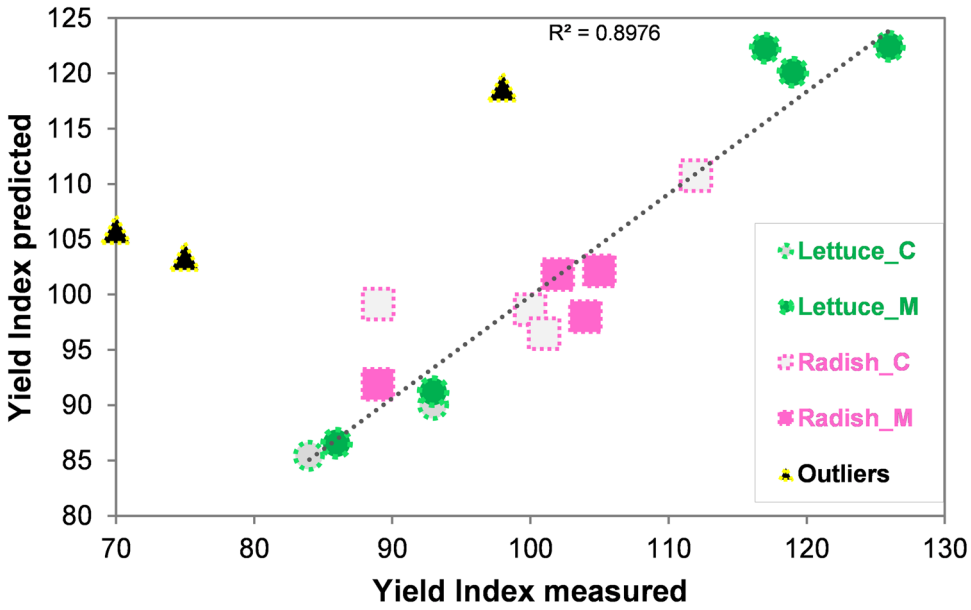

Fig. 4 Scatterplot of the measured vs. predicted yield index from the average spectra of the UNISG-18 experiment. Wide symbols are control non-inoculated (*_C*), and solid symbols are microbial inoculated plots (*_M*). Three outliers are shown in triangles

Figure 8 reports the average reflectance spectrum of the litterbags after the 1st derivative math treatment, which shows how the sine waves appear after 990 nm. In fact, the Pearson correlation coefficients, which are reported in Fig. 9, assumed higher values after such a wavelength.

3.3 Litterbag-NIRS Variables and Association with the Microbial Inoculation and the Yield Index

The average values for the control and microbial groups and standard deviation of the 22 LBN variables, paired with the mean composition of the hay, which remained stable over the three trials, are reported in Table 5. Significant increases in the soil respiration (+5%), litter crop maturity index (+4.5%), indigestible NDF (+0.8%), and ether extract (+0.4%) were observed in the microbially inoculated parcels, while the NDF digestibility (−0.8%) and the digestible NDF (−0.9%) were reduced. A significant rise in variances (21÷31%) was observed in a Levene test for the SIR, NO_3^-N, and N-Free extract of the microbial groups.

A certain homogeneity of the standardized coefficients was exhibited for the partial least squares LBN models of the yield index, observed in the 13 theses (Table 6). The most positive yield features resulted in a NH_4^+-N level of the soil and a higher hemicellulose content with better NDF digestibility of the litters. On the other hand, the most unfavorable traits in the litterbags were assigned to the lignin and crude protein contents, which were associated with an excess of the putative microbial r-strategist populations.

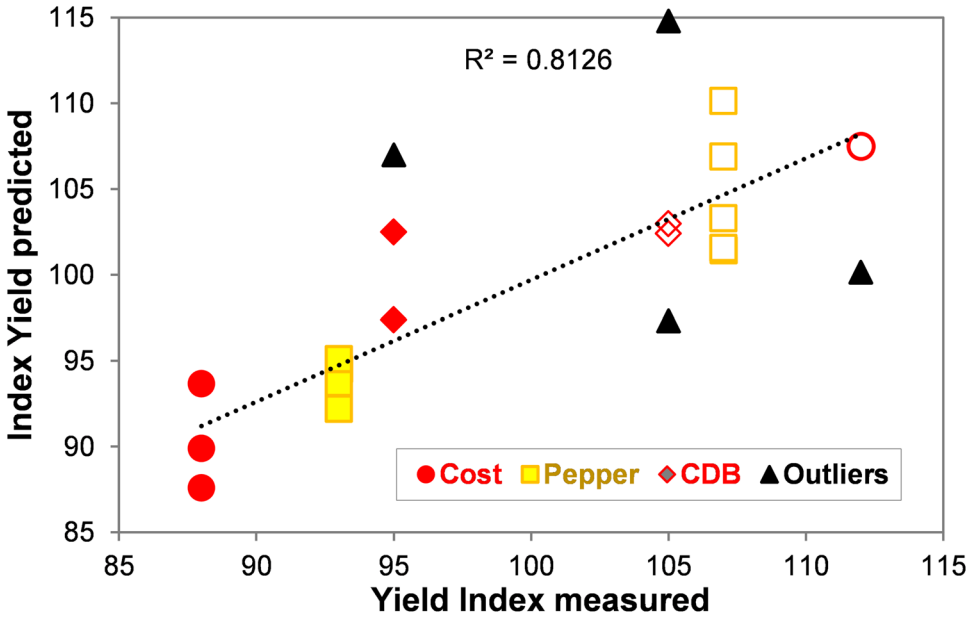

Fig. 5 Scatterplot of the measured vs. predicted yield index from average spectra of the UNISG-19 experiment. *Cost* tomato Costoluto, *Pepper* bell pepper, *CDB* tomato Cuore di Bue, wide symbols are control non-inoculated, and solid symbols are microbial inoculated plots. Four outliers in triangles are shown

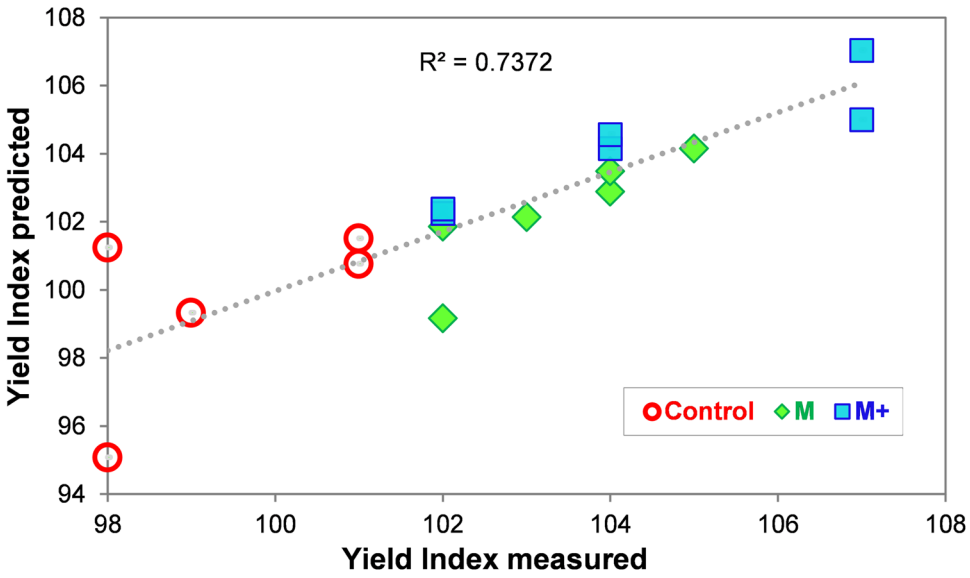

Fig. 6 Scatterplot of the measured vs. predicted yield index from average spectra of the SERMIG-20 experiment. Wide symbols control non-inoculated, solid symbols microbial inoculated plots, with single dose (M) or double dose (M+)

Table.4 Calibration and cross-validation of the yield index, of the microbial inoculation in the three experiments and on the average NIR spectra of the 61 groups, and of the year on the single spectra

		Yield index										Microbial treatment	
Experiment	Method	No	Mean	SD	SECV	R^2	RPD	SECV/mean %	Math	nm1	nm2	No	R^2
P18	MPLS	15	101.3	12.77	7.89	0.66	1.62	7.8	1,2,2			14	0.47
P19	MPLS	17	99.0	8.17	5.41	0.58	1.51	5.5	1,2,2			19	0.27
S20	MPLS	17	100.3	3.2	1.7	0.73	1.95	1.6	1,2,2			17	0.80
Pool	MPLS	10	98.85	6.38	4.71	0.55	1.36	4.8	1,2,2			10	0.17
Pool	Stepwise regression	12	99.51	6.86	3.76	0.67	1.82	3.8	0,1,1	1031	986		
Year (1, 2, 3)	MPLS	345		0.77	0.28	0.87			1,2,2				

SD standard deviation, *SECV* standard error in cross-validation, R^2 r-square in CV, *RPD* residual predictive deviation = SD/SEC, *Math* pretreatment of the spectra (derivative, smoot1, smooth2), *nm1,2* wavelengths of maximum response, *MPLS* modified partial least squares

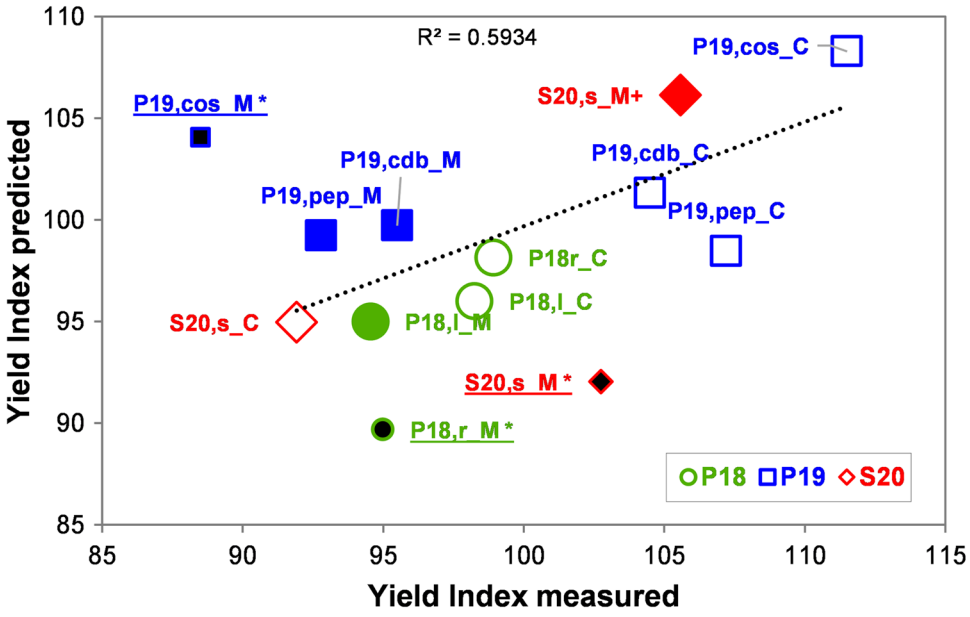

Fig. 7 Scatterplot of the measured vs. predicted yield indexes' theses from the average spectra of the pooled experiments. (i) P18, P19, S mean the three trials; (ii) code of the crops: l lettuce, r radish, cos tomato *Costoluto*, cdb tomato *Cuore di Bue*, pep bell pepper, S scarola; (iii) inoculation: _C control non-inoculated, _M and M + (double inoculation) with solid symbols microbial inoculated theses, three outliers are noted * and outlined

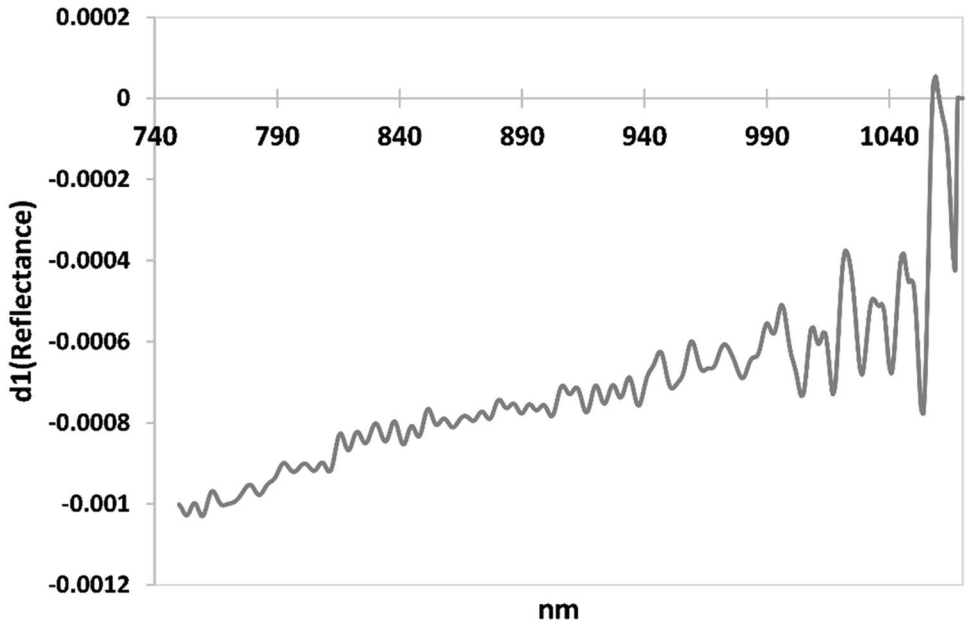

Fig. 8 Average reflectance spectrum of the litterbags after 1st derivative

4 Discussion

As shown in the second UNISG trial (Oggiano et al. 2021), the lack of any quantitative effect of the inoculation may have been due to a particular hostility of the autochthonous microflora in the organic soil, which acted as a biotic impediment. Although the luxuriant effect was absent, the foliar pH and the NIR spectroscopy examination of the leaves, roots, and fruit showed that the inoculation of the microbial consortium had qualitatively modified the plants and fruit to a certain extent, and this modification was recognized by an expert sensory panel. The first trial was carried out on a peat substrate, which was probably very poor in microflora, and thus unable to trigger and maintain a favorable hospitable environment. The pseudo-sterile conditions adopted in many experiments with biofertilizers require the utilization of very high doses of inocula before any significant effects can be observed. Under real field conditions, when it is necessary to deal with autochthonous soil microflora, which are as numerous as they are unknown, it has been verified that very low doses, such as that calculated for 71 spores of *Rhizophagus irregularis* per potato seed over 231 real fields (Hijri 2016), can

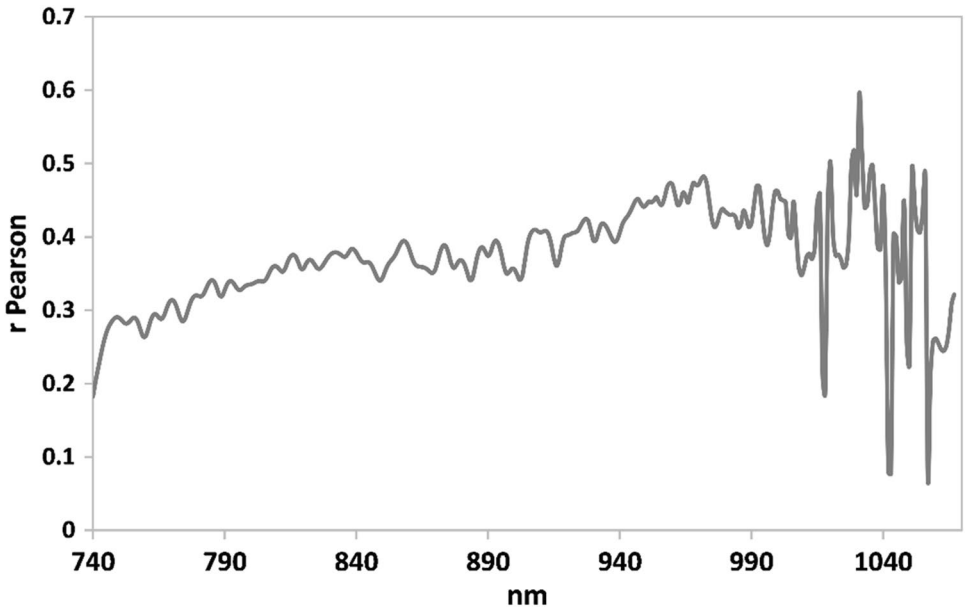

Fig. 9 Coefficients of the Pearson correlation of the yield index on the 1st derivative reflectance spectrum of the litterbags

Table.5 Litterbag-NIRS (LBN) variables means in control and microbial treatments, change in standard deviation, and hay means

Litterbag-NIRS (LBN) variable	Acronym	Unit	C Control	M Microbial	SD	dSD %	Hay means
Ash	Ash	%DM[1]	19.33	19.45	2.11	0	6.05
Crude protein	CP	%DM	13.13	13.26	1.06	−7	3.28
NDF	NDF	%DM	45.07	45.04	0.22	6	45.02
Digestible NDF	DNDF	%DM	24.21a	24.01b	0.81	−2	30.4
Indigestible NDF	INDF	%DM	20.85b	21.03a	0.81	0	14.62
ADF	ADF	%DM	26.42	26.08	3.67	−2	37.96
Gross energy	GE	MJ/kg	15.75	15.74	0.15	5	16.95
In vitro total digestibility	IVTD	%	79.97	79.99	1.81	−4	67.58
NDF digestibility	NDFD	%	53.72a	53.30b	1.78	−1	67.52
Crop maturity index	CMI	n	0.874b	0.914a	0.11	4	0.45
Lignin	ADL	%DM	6.616	6.561	1.13	9	13.03
Cellulose	Cell	%DM	19.80	19.52	2.73	−3	24.44
Crude fiber	CF	%DM	11.40	11.46	3.25	10	21.54
Ether extract	EE	%DM	2.85b	2.86a	0.05	3	2.98
Hemicellulose	Hemic	%DM	27.30	27.21	3.29	−1	7.06
N-free extract	NFE	%DM	49.17	49.12	0.63	21 *	45.92
Soil traits							
NH_4^+-N	NH_4	mg kg^{-1} DM	4.628	4.626	1.22	8	
NO_3^--N	NO_3	mg kg^{-1} DM	55.22	57.62	13.09	31 *	
Microbial traits							
Substrate induced respiration	SIR	µg Cmic g^{-1}FW	109.2b	114.7a	19.03	29 *	
r-strategists fingerprinting	r	%	5.742	5.851	1.52	1	
k-strategists fingerprinting	k	%	7.332	7.376	0.9	−2	
r-k prevalence	r-k	%	−1.59	−1.52	0.72	12	

DM dry matter, *SD* total standard deviation, *dSD* (SD(M)/SD(C)-1)*100

Table.6 Partial least squares (PLS) coefficients in Litterbag-NIRS (LBN) models for yield index, within experiments and pooled. The LBN variables are ordered by the mean value of the PLS coefficients. The last column refers to the trial effects

LBN-variable	Acronym	Experiment			Pool	Mean PLS coefficient
		P18	P19	S20		
Soil NH_4^+-N	NH4	3.441	1.625	2.010	1.235	2.078
Hemicellulose	Hemic	0.548	3.188	1.120	−2.289	0.642
NDF digestibility	NDFD	3.080	−1.071	0.961	−0.553	0.604
ADF	ADF	−1.469	1.842	−0.504	1.736	0.401
Digestible NDF	DNDF	1.955	−0.427	0.344	−0.295	0.394
Total digestibility	IVTD	0.812	−0.103	0.304	0.293	0.327
Cellulose	Cell	−1.141	−0.061	−0.129	2.604	0.318
NDF	NDF	−0.922	0.492	0.616	1.084	0.317
Soil substrate induced respiration	SIR	1.282	−0.151	−0.434	0.009	0.176
Crude fiber	CF	0.178	−2.034	4.944	−2.498	0.148
Ash	Ash	0.083	0.468	−1.166	1.111	0.124
Gross energy	GE	−0.378	0.111	0.103	0.376	0.053
Ether extract	EE	−0.430	0.062	0.356	0.129	0.029
Indigestible NDF	INDF	−2.877	0.918	0.272	1.379	−0.077
Microbial k-strategists	k	0.418	−0.078	−0.329	−0.344	−0.083
Crop maturity index	CMI	−0.223	−0.037	−0.081	−0.012	−0.088
Microbial r to k difference	r-k	−1.730	0.206	0.151	0.706	−0.167
N-Free Extract	NFE	−2.314	0.329	0.065	1.166	−0.189
Soil NO_3^--N	NO3	−1.224	1.185	−0.081	−0.839	−0.240
Microbial r-strategists	r	−1.312	0.128	−0.178	0.362	−0.250
Crude protein	CP	0.613	−0.958	−0.047	−0.948	−0.335
Lignin	ADL	−0.328	−0.913	−0.375	−0.868	−0.621
R^2 (PLS model)	R^2	0.392	0.511	0.257	0.742	
Standard deviation	SD	13.268	6.387	9.856	3.859	
Mean squares error	MSE	127	31.08	70.15	7.45	
Residual MSE	RMSE	11.275	5.575	8.376	2.729	

Pool pool of the three experiments, *PLS* partial least squares, *SD* standard deviation

find a hospitable environment and collaborate in developing an effective and useful symbiosis.

According to some literature reports on biofertilizers (Schütz et al. 2017), the luxuriance effects on yield have mainly been achieved for legumes (+19%), vegetables (+17%), cereals (+15%), and roots (+10%). However, interactive patterns have been described in literature as a result of reactions to autochthonous soil microflora (Klironomos 2003; Regvar et al. 2003; Hart et al. 2018) and significant interactions have been observed for saffron, between the cultivation year and mycorrhizal treatments, for the yields, flowers, and shoot size (Caser et al. 2019).

In the first report of litterbags on six different crops (Masoero et al. 2018), microbial inoculation resulted in a significant increase in the ether extract of the litterbags but did not result in any concordant significant positive variations in the protein, crude fiber, and ash, or any negative variations in ADF, DNDF, NDF, cell, and NFE. The most discordant components were the crop maturity index, INDF, NDFD, Hemic, and ADL. However, the proportion of the correct classification of the previous work in the control (62%) and in microbial treatments (71%) is in agreement with the 65% and 73% of the present work. In fact, it is normal for a control to be characterized less and therefore be less recognizable than an inoculated treatment: this could be a first indirect indication of an efficient inoculation by the Litterbag-NIRS method.

As it is well known that there are no substance peaks in NIR radiation, but only resonances and overtones from the vibration interactions of the organic molecules that are located in the IR region, it is interesting to observe that high correlations occurred between the reflectance spectra of the litterbags and the production index in the 1031 and 986 nm bands. The relationships were revealed after the first derivative of the spectra, thus emphasizing the need to measure continuous fractions of the spectrum and not only to have information obtained from single points: the PLS coefficients were high in numerous other points, and all the information can be used in the multivariate model. It should be pointed out that this region is generally below the range of NIRS bench devices.

Quantitative litterbags are usually adopted to characterize the transformation of litter over a natural cycle, but not for agricultural crops. Only a handful of studies that have used plant litter to test decomposition on a global scale (Parton et al. 2007) have shown that the combination of temperature and moisture can help explain 50–70% of the decomposition variation. The Tea Bag Index, which was formulated by the agroecologists Keuskamp et al. (2013), is a novel approach that can be used to collect uniform decomposition data across ecosystems. The method uses green tea leaves, which are more decomposable and suitable to fix the lower bound of the time decay (S-Stabilization), and red tea leaves, which are more resistant and thus more suitable to fit the usual parameter of the decomposition rate constant (k), in paired tetrahedron-shaped synthetic litterbags. Didion et al. (2016), for harmonization purposes, assessed the decomposition decay rate and the stabilization factor, for a short term of 60 days, obtained from standardized litter experiments, because they could provide a key prerequisite to further develop simulation models for the estimation of the C balance of ecosystem litter pools.

The NIRS of decaying forest foliage was first studied in 1991 (McLellan et al.) then by Gillon et al. (1999). During the decay process, a broad absorbance feature develops in the 1100–2000 nm region of the near infrared spectrum. The magnitude of this feature is related to the age of the material (or to the degree of decomposition) and may be useful to determine the degree of decay of field samples. The chemistry of litter can explain the contrasting feeding preferences of bacteria, fungi, and higher plants (Bonanomi et al. 2017). Plant litter has specific and contrasting effects on bacteria, fungi, and higher plants, thus highlighting that different microbial food web components should be investigated simultaneously to understand the effects of plant detritus on the structure and functionality of an ecosystem. An interesting study on litter investigation is that of Gioacchini et al. (2015) who, by means of thermogravimetry–differential thermal analysis, showed weak correlations with cellulose and lignin measured by means of a chemical method. When the cell walls extracted from the litter were examined, a good correlation was found between the two methods for cellulose and lignin.

Direct attempts to search for substance markers of mycorrhizal symbiosis have been made in soil as well as in leaves. Until now, the approaches available to measure and quantify mycorrhizal-plant associations have required excavation of the roots, followed by microscopic and transcript analyses. However, these methods are impractical for HTP screening, due to the damage caused by root sampling, and are laborious for the quantification of fungal fatty acids. Hence, an HTP screening technique is needed to empower research and development in breeding programs to obtain improved mycorrhizal-plant associations. According to Watt et al. (2006), new techniques that act on the biotas are available and they allow a direct visualization and quantification of the rhizosphere processes under field conditions (Ryan et al. 2003). Biosensors are searched to identify specific substances in the soil, such as the antibiotics released by *fluorescens* pseudomonad (Hansen et al. 2021). Wang et al. (2018) found that a group of molecules, called blumenols, accumulate in the roots, shoots, and leaves of tomato, potato, and barley plants as a result of effective symbiosis with mycorrhizal fungi. Measuring the levels of blumenols in plant shoots and leaves is much quicker and easier than the current methods used to identify fungal symbioses in plant root samples. Therefore, measuring blumenols may be a useful way for plant breeders to screen large numbers of plants to establish the degree of symbioses, and to breed crops that manage better interactions with the beneficial fungi. Most of the candidate genes for blumenol biosynthesis are upregulated in the roots, but not in the leaves. The chemical extraction procedure for the HTP quantification of blumenol derivatives in leaves, in both model plants and economically relevant crops, was described in Mindt et al. (2019).

The first reference to a yield prediction from NIR spectra was achieved in a wine trial (Cugnetto et al. 2021) in which an R^2 cross-validated value of 0.91 was found for *Nebbiolo* and 0.67 for *Erbaluce* vines. In a previous study on tomatoes (Baldi et al. 2020), a linear regression of the yield on the % fingerprinting of the treatments with biofertilizer was observed in the NIRS of the litterbags, which reached an R^2 of 0.45, but when the NIRS fingerprinting of the leaves was added, the bilinear regression reached an R^2 of 0.95. In a study on the use of biofertilizers on potatoes (Volpato et al. 2020), the NIR spectra of litterbags allowed a model to be achieved with an R^2 of 0.67 for an average of eight sub-groups. Further interesting information was obtained for maize (Volpato et al. 2021), where a parabolic function, with an R^2 of 0.37, connected the fingerprinting % of the yield response to a biofertilizer treatment.

The usual greenhouse crop yield prediction models are used to manage specific application scenarios, but this may not ensure accuracy of the results if the greenhouse environment changes. The two frequently used tomato growth models, TOMGRO and Vanthoor, allow prediction errors of around $7 \pm 1\%$ and $12 \pm 6\%$, respectively, to be obtained (Lin et al. 2019). However, the greenhouse environment is a complex system with multi-variables, as well as nonlinearity of the transpiration and photosynthesis, and growth models have in fact received somewhat limited attention, even though their precision is convenable. The lack of smart growth models has led to insignificant increases in crop yields and unsatisfactory control effects in greenhouse microclimates. Therefore, there is an urgent need for a more

versatile and applicable crop yield model. Ehret et al. (2011) performed neural network modeling of the yield, growth, and water use of greenhouse tomatoes through passive automated crop monitoring (radiation, temperature, rain) as well as from active data (water, CO_2 fertilization). Yield was found to be more closely related to radiation from the previous week ($R^2 = 0.65$) than to radiation in the current week ($R^2 = 0.56$).

The microbial profiles of litterbags and of soil can be quite different, as first shown in the aforementioned study of Baldi et al (2021), and any microbial inference about the LBN variables should therefore be limited to the litterbag-sphere, which is an attractive cornucopia for the hyphae as well as for the roots of the neighboring crops, and for the water-moved microorganisms. The importance of the soil microbes in heterosis has largely been ignored. A first study by Wagner et al. (2021) has shown that the heterosis of the root biomass and other traits in maize depends to a great extent on the belowground microbial environmental, although the intrinsic profile of the natural soil can drive the responses in different ways. All this underlines the importance of microbiologically defining the soil that acts as a control group in each experiment that involves microbial effectors.

For the present method to succeed, it is sufficient to ensure that an adequate number of litterbags are distributed throughout the fields.

At the end of the work, we found a rapid, indirect, low-cost method that has existed and has been used since 1975 (Doran et al. 1997) by the Wood End Research Institute (Mt Vernon, ME, USA).

5 Conclusions

The use of qualitative litterbags, combined with low-cost spectrometers, has opened a new panorama in the field of agricultural investigations. In the present experiments, the litterbag-NIRS method has been used to forecast production, but also to reveal some of the factors that may be of benefit to support production.

First, the type of crops which, with the same soil, irrigation, etc., modify the rhizosphere has been detected clearly and significantly by means of the Litterbag-NIRS method. This is the first time that it has been ascertained that soil changes according to the different crops in an indirect and simple way: one soil can produce various effects on the litterbags from different crops.

Litterbag-NIRS was created to interpret the response of plants to the use of biofertilizers. This is not a real-time effector and cannot therefore be used to solve problems related to active growth regulation, especially in a highly technological field, but it can be used to trace some of the complex relationship mechanisms that plants trigger in the rhizosphere, whether in the soil or substrate, pertaining to their nutrition and ontogenetic development. The best possible answer to such an enigma, perhaps some months in advance, is an unbiased prediction of the production that will be harvested.

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Declarations

Conflict of Interest The authors declare no competing interests.

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