Soil management intensity shifts microbial catabolic profiles across a range of European long-term field experiments

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ABSTRACT

Assessing soil microbial functionality has the potential to reveal meaningful effects of soil management on soil processes influencing soil quality. We used MicroResp™ to assess microbial respiration upon the addition of six carbon substrates (glucose, alanine, aminobutyric acid, *N*-acetyl glucosamine, alpha-ketoglutaric acid, and lignin). From this, we calculated the multiple substrate induced respiration (MSIR), the microbial catabolic profile expressed as absolute and relative utilization rate, and the Shannon microbial functional diversity index (H′). We tested the effect of tillage (*reduced* vs. *conventional*) and organic matter addition (*high* vs. *low*) on these microbial parameters in soil from 10 European long-term field experiments (LTEs), and investigated their relationships with labile organic carbon fractions and various soil parameters linked to soil functions. Reduced tillage and high organic matter input increased MSIR compared to conventional tillage and low organic matter input. In addition, reduced tillage resulted in a small but significant increase in functional diversity compared to conventional tillage. An increase in soil management intensity (CT-Low > CT-High > RT-Low > RT-High) was associated with lower utilization of all the substrates expressed as absolute utilization rate, and a proportionately higher utilization of alpha-ketoglutaric acid compared to the other substrates. More intensive management systems also showed lower soil quality as measured by various soil parameters, in particular total and labile organic carbon, basal respiration, and microbial biomass nitrogen. The present work shows for the first time the key role of labile organic carbon, as affected by soil management, in determining microbial functional diversity. Aggregating results from 10 European arable LTEs, making use of a comprehensive dataset, MicroResp™ showed that reduced tillage and increased organic matter addition created a more favourable habitat for the microbial community to utilize different carbon substrates and, thereby, the potential for nutrient cycling.

1. Introduction

Soil microbial communities have a primary role in various soil processes such as nutrient cycling, decomposition, carbon sequestration, soil structure development, water cycling and retention, and control of pest and pathogen populations (Barrios, 2007; Murphy et al., 2007). Since soil microorganisms and the processes they perform are sensitive to chemical and physical changes in their environment, they can be used to monitor the effects of soil management on soil functioning (Bünemann et al., 2006). Ultimately, changes in soil microbial properties caused by agricultural management can inform about changes in soil quality, defined as the capacity of soil to perform multiple functions (Bünemann et al., 2018).

Microbial parameters have been included in soil quality assessment schemes since many years, but they are less frequently used than chemical and physical indicators (Bünemann et al., 2018; Schloter et al., 2018). For soil quality assessment, measuring microbial functionality, i.e. the type of processes that the microorganisms can carry out, and to what extent these processes are carried out (e.g. decomposition and nitrogen mineralization), can be more informative than the assessment of microbial biomass, the presence of individual or groups of organisms and/or community composition based on purely taxonomic information (Krause et al., 2014; Wood et al., 2015b; Zak et al., 1994). Several studies found that changes in functional diversity could more successfully be used to understand effects of land use on soil microbial communities and related soil processes than changes in taxonomic diversity

([Bei et al., 2018](#); [Cheng-yu et al., 2018](#); [Manoharan et al., 2017](#); [Wood et al., 2015a](#)).

Community level physiological profiling (CLPP), also called catabolic profiling, is a method used for the study of soil microbial functional diversity. This method quantifies the functional diversity of the microbial community by monitoring its potential to decompose a selection of carbon substrates with contrasting chemical characteristics, i.e. carbohydrates, amines, amino-acids, carboxylic acids and more recalcitrant compounds such as polymers or phenolic compounds ([Campbell et al., 2003](#)). The advantages of this method over other methods for measuring microbial functionality is that it relies on the assessment of the microbial community active in decomposition processes relevant in agricultural soils. In addition, it is relatively simple and fast to perform, analyse and interpret, gives information about microbial abundance, decomposition rates, microbial preferences for specific substrates and, based on this last information, microbial functional diversity. One of the most widely applied CLPP systems is MicroResp™ ([Campbell et al., 2003](#)). MicroResp™ has been used for determining differences in microbial functional diversity due to land use ([Brackin et al., 2013](#); [Creamer et al., 2016](#); [Moscatelli et al., 2018](#); [Murugan et al., 2014](#)) and to soil management practices such as mineral fertilization and organic matter addition ([Ge et al., 2013](#); [Hupfauf et al., 2016](#); [Martínez-García et al., 2018](#); [Murugan et al., 2014](#); [Pan et al., 2016](#); [van der Bom et al., 2018](#)) and, to a lower extent, tillage ([Rincon-Florez et al., 2016](#); [Zhang et al., 2018](#)). Organic matter addition can increase microbial biomass and alter the microbial community composition, steering it towards higher abundance and activity especially of those organisms that can degrade a wide variety of substrates ([Ge et al., 2013](#); [Martínez-García et al., 2018](#); [van der Bom et al., 2018](#)) and recalcitrant substances ([Francioli et al., 2016](#); [Hartmann et al., 2014](#)), thereby enhancing microbial functional diversity ([Gomez et al., 2006](#); [Govaerts et al., 2007](#); [Murugan et al., 2014](#); [Nair and Ngouajio, 2012](#); [Reilly et al., 2013](#)). Also reduced tillage generally affects microbial community composition and increases microbial activity and metabolic capacity in the topsoil ([Hao et al., 2019](#); [Mbuthia et al., 2015](#)).

Higher soil total organic carbon levels have been found to correspond to higher overall microbial catabolic diversity ([Degens et al., 2000](#); [Nsabimana et al., 2004](#)) and substrate utilization efficiency ([Creamer et al., 2016](#); [Francioli et al., 2016](#); [Zhong and Cai, 2007](#)). However, not only the quantity but also the quality of organic matter is crucial for microbial activity, diversity and the relative abundance of different microbial functional groups ([Bending et al., 2002](#); [Degens et al., 2000](#); [Gupta and Germida, 2015](#); [Huang et al., 2008](#); [Lagomarsino et al., 2012](#)), and previous studies reported a positive relationship between microbial functional diversity and different labile carbon fractions (HWEC and DOC) ([Huang et al., 2008](#); [Tian et al., 2015](#)). These relationships can be explained by the general increase of more easily decomposable organic carbon fractions in less intensive management practices (such as reduced tillage and organic fertilization) and the consequent gain in catabolic diversity ([Graham and Haynes, 2005](#)).

Despite the potential role of microbial catabolic profiles in revealing effects of soil management on microbial functions related with decomposition and nutrient cycling, knowledge is lacking about the combined effect of long-term tillage and organic matter addition on microbial catabolic profiles, and the relation with soil parameters, in particular labile organic carbon. Our study is the first one to address these questions, expanding our understanding of the drivers of CLPP by using a range of European long-term field experiments. This comprehensive assessment has the potential to elucidate if the microbial catabolic profile is a suitable indicator for soil quality in arable systems.

We measured the response of the catabolic profile to tillage and organic matter addition in soil samples from ten European arable long-term field experiments located in different pedoclimatic zones. Thereafter, we related the MicroResp™ results with various labile carbon fractions and with an extensive set of soil quality parameters

that were measured in the same samples to understand the consequences of agricultural management for soil functioning and to improve our understanding of these relationships. The specific objectives of our study were to i) determine if tillage (reduced vs. conventional), organic matter addition (high vs. low) and their interaction affect basal respiration, multiple substrate induced respiration and catabolic profiles, ii) identify how the utilization of carbon substrates discriminates between the different soil management practices, and which are the most important substrates contributing to this separation, iii) determine if and how soil management affects microbial functional diversity; and iv) assess which are the most important parameters driving the catabolic profiles and microbial functional diversity.

We hypothesised that reduced tillage and increased organic matter addition, considered as less intensive soil management, will i) stimulate microbial activity and result in consistent differences in catabolic profiles, ii) show higher utilization of more recalcitrant substrates such as lignin and, generally, a more even substrate utilization, iii) increase microbial functional diversity; and iv) we hypothesised that the structure of the catabolic profiles and the functional diversity under less intensive soil management will be driven by labile organic carbon.

2. Materials and methods

2.1. Long-term field experiments, management and soil sampling

Ten European long-term field experiments (LTEs) with different pedoclimatic characteristics were sampled in spring 2016 before any soil management was applied (Table S1). Each LTE had unique management characteristics and a different experimental design, with three or four replicates per treatment. Despite their uniqueness, the main soil management types (i.e. tillage and organic matter addition) were in common between the LTEs, making them comparable ([Bongiorno et al., 2019a](#); [Bongiorno et al., 2019b](#); [Bongiorno et al., 2019c](#)). The contrast in tillage was classified as conventional tillage (ploughing at 20–25 cm depth, CT) versus reduced tillage (no-tillage or non-inversion tillage at 0–10 cm, RT). The contrast in organic matter addition was classified as low organic matter addition (Low, no organic matter addition or only mineral fertilization) versus high organic matter addition (High, organic matter additions with or without mineral fertilizer). In some LTEs, both treatment factors were applied, while at others only one of these was present.

In spring 2016, soil samples were collected from the LTEs before any major soil or crop management was applied. Twenty soil cores were randomly collected in the central area of the plot with 3 cm diameter augers, to avoid border effects, and mixed to provide one composite soil sample per treatment and replicate at each site (total of 167 samples). In seven trials (CH1, CH2, NL1, NL2, SL1, HU4 and ES4) two layers (0–10 cm and 10–20 cm) were sampled because tillage was part of the soil management. In three trials (CH3, PT1 and HU1) only one layer (0–20 cm) was sampled because the only management factor was organic matter addition and we did not expect a stratification effect due to tillage. In the current study we used the samples from the 0–10 cm layer for CH1, CH2, NL1, NL2, SL1, HU4, ES4 and the 0–20 cm layer samples for CH3, PT1 and HU1, for a total of 101 samples (the same samples as used in [Bongiorno et al., 2019c](#)). Fresh soil samples were sent to Wageningen University (The Netherlands), Research Institute of Organic Agriculture (Frick, Switzerland), University of Trier (Germany) and University Miguel Hernandez (Alicante, Spain), and air-dried samples were sent to University of Ljubljana (Slovenia). Upon arrival, the samples were sieved at 5 mm and, when fresh, stored at 3 °C until further processing.

2.2. Chemical, physical and biological soil parameters

Various soil properties were measured for the current study: total organic carbon in soil (TOC; %), pH (CaCl$_2$), total nitrogen (TN; %),

Table 1

Overview of methods used to determine chemical, physical, and biological parameters linked with soil processes and the methods used to measure labile carbon fractions, soil suppressiveness and nematode community characteristics (Bongiorno et al., 2019a; Bongiorno et al., 2019b; Bongiorno et al., 2019c).

Parameters	Methodology	Unit	Laboratory of analysis
Chemical parameters			
Total organic carbon (TOC)	SIST ISO 10694: Soil quality - Determination of organic and total carbon after dry combustion ("elementary analysis")	%	University of Ljubljana (SL)
Total nitrogen (TN)	SIST ISO 13878:1999: Soil quality - Determination of total nitrogen content by dry combustion ("elementary analysis")	%	University of Ljubljana (SL)
pH	$CaCl_2$ determination- SIST ISO 10390:2006: Soil quality - Determination of pH	–	University of Ljubljana (SL)
Cation exchange capacity (CEC)	ISO 13536:1995: Soil quality - Determination of the potential cation exchange capacity and exchangeable cations using barium chloride solution buffered at pH = 8,1	$cmol_c\ kg^{-1}$ soil	University of Ljubljana (SL)
Plant available phosphorus (P_2O_5)	ÖNORM L 1087 - modification: ammonium lactate extraction	$mg\ kg^{-1}$ soil	University of Ljubljana (SL)
Plant available potassium (K_2O)	ÖNORM L 1087 - modification: ammonium lactate extraction	$mg\ kg^{-1}$ soil	University of Ljubljana (SL)
Exchangeable magnesium, calcium, and sodium (Mg^{2+}, Ca^{2+}, Na^+)	ammonium acetate extraction; Soil survey laboratory methods manual, 1992	$mg\ kg^{-1}$ soil	University of Ljubljana (SL)
Physical parameters			
Water stable aggregates (WSA)	Wet sieving method modified as in Öhlinger and Kandeler (1996)	$mg\ kg^{-1}$ soil	FiBL (CH)
Bulk density (BD)	Volumetric assessment with ring	$g\ cm^{-3}$	Field assessment by LTE owners
Silt, clay and sand	SIST ISO 11277:2011: Soil quality - Determination of particle size distribution in mineral soil material - Method by sieving and sedimentation	%	University of Ljubljana (SL)
Water holding capacity (WHC)	Calculated with a pedotransfer function using the % clay, silt and total organic carbon (Tóth et al., 2015)	%	Wageningen University & Research (NL)
Biological parameters			
Microbial biomass carbon (MBC)	Fumigation extraction method (Vance et al., 1987)	$mg\ kg^{-1}$ soil	Trier University (DE)
Microbial biomass nitrogen (MBN)	Fumigation extraction method (Vance et al., 1987)	$mg\ kg^{-1}$ soil	Trier University (DE)
Soil respiration	Incubation of soil at 25 °C for 72 h in thermostat bath	$\mu g\ CO_2 -C\ h^{-1}\ g^{-1}$ soil	University Miguel Hernandez (ES)
Earthworms abundance and biomass	Hand sorting from $30*30*30\ cm^3$ monolith	Number and fresh weight ($g\ m^{-2}$)	Field assessment by LTE owners
Tea bag decomposition	Tea bag incubation (tea bag index) (Keuskamp et al., 2013)	% mass loss	Field assessment by LTE owners
Soil suppressiveness to *Pythium ultimum*	*Pythium ultimum*-cress bioassay (Tamm et al., 2010)	Soil suppressiveness	Wageningen University & Research (NL)
Nematode abundance	qPCR quantification (Bongiorno et al., 2019a)	qPCR DNA counts 100 g^{-1} soil	Wageningen University & Research (NL)
Nematode richness and diversity	18S DNA Sequencing (Bongiorno et al., 2019a)	OTU numbers and diversity (exp^H)	Wageningen University & Research (NL)
Labile carbon fractions			
Dissolved organic carbon (DOC)	Extraction with ultrapure water and filtration at 0.45 μm filters.	$mg\ kg^{-1}$ soil	Wageningen University & Research (NL)
Hydrophilic dissolved organic carbon (Hy-DOC)	Fractionation of DOC with DAX-8 resin (Van Zomeren and Comans, 2007).	$mg\ kg^{-1}$ soil	Wageningen University & Research (NL)
Dissolved organic carbon and hydrophilic dissolved organic carbon specific ultraviolet absorbance (DOC SUVA and Hy SUVA)	Analysis of DOC and Hy solution with spectrophotometer at 254 nm (Weishaar et al., 2003; Amery et al., 2008).	$L\ g\ C^{-1}\ cm^{-1}$	Wageningen University& Research (NL)
Permanganate oxidizable carbon (POXC)	Oxidation with K_2MnO_4 (Weil et al., 2003).	$mg\ kg^{-1}$ soil	Wageningen University & Research (NL)
Hot water extractable carbon (HWEC)	Extraction with hot water (80 °C) for 16 h and filtration at 0.45 μm filters (Ghani et al., 2003).	$mg\ kg^{-1}$ soil	Wageningen University & Research (NL)
Particulate organic matter carbon (POMC)	Suspension in NaCl for 15 h, wet-sieving through a 53 μm sieve and calculation of POM by loss on ignition (Salas et al., 2003).	$mg\ kg^{-1}$ soil	FiBL (CH)

cation exchange capacity (CEC; $cmol_c\ kg^{-1}$ soil), extractable phosphorus by the Olsen method (P; $mg\ kg^{-1}$ soil), plant available potassium (K; $mg\ kg^{-1}$ soil), exchangeable magnesium, calcium and sodium (Mg^{2+}, Ca^{2+}, Na^+; $mg\ kg^{-1}$ soil), water stable aggregates (WSA; $mg\ kg^{-1}$ soil), water holding capacity (WHC; %), bulk density (BD; $g\ cm^{-3}$), percentages of silt, clay and sand, microbial biomass carbon (MBC; $mg\ kg^{-1}$ soil), microbial biomass nitrogen (MBN; $mg\ kg^{-1}$ soil), number and biomass of earthworms (number and $g\ m^{-2}$), decomposition through tea bag index (% mass loss), soil suppressiveness to *Pythium ultimum* (%) (Bongiorno et al., 2019c), nematode abundance (DNA counts 100 g^{-1} soil), and nematode OTU richness and diversity (Bongiorno et al., 2019a). Five labile carbon fractions were measured as explained in Bongiorno et al. (2019b): hydrophilic dissolved organic carbon (Hy-DOC; $mg\ kg^{-1}$ soil), dissolved organic carbon (DOC; $mg\ kg^{-1}$ soil), permanganate oxidizable carbon (POXC; $mg\ kg^{-1}$ soil), hot water extractable carbon (HWEC; $mg\ kg^{-1}$ soil), and particulate organic matter carbon (POMC; $mg\ kg^{-1}$ soil). The recalcitrance of Hy-DOC and DOC was assessed measuring their specific ultraviolet absorbance (Hy SUVA and DOC SUVA; $L\ g\ C^{-1}\ cm^{-1}$). For details about the methods used for assessing these parameters we refer to Table 1.

2.3. Community level physiological profiling

For the community level physiological profiling (catabolic profiling) we used the MicroResp™ system, as done by Campbell et al. (2003) and Creamer et al. (2016). Briefly, the colorimetric gel detection plates were prepared by mixing 150 μl of noble agar and a pH indicator solution containing 12.4 ppm, wt/wt cresol red, 150 mM KCL and 2.5 m M NaHCO₃. Subsequently, 125 μl of indicator dye was transferred to each well of the detection plate.

The soils were sieved at 2 mm, added to the deep-well plates using the MicroResp™ filling device and incubated for 6 days at 25 °C between 30 and 60% of their WHC, following the MicroResp™ technical manual (Cameron, 2007) and Creamer et al. (2016), to minimize artefacts associated with soil disturbance induced by sampling and sieving on the microbial community.

We used eight substrates to produce the respiration rates: glucose (G; as simple sugar), alanine and gamma-amino butyric acid (A and AMA; as amino acids), n-acetyl-glucosamine (NAC; as amide), oxalic acid and alpha-ketoglutaric acid (OA and AKA; as carboxylic acids) and lignin (L; as polymer). In addition, we used deionized water as control. These substrates were selected because of their biological relevance in agriculture being components of root exudates, microbes or end products of plants and microbes (Murugan et al., 2014) and because they represented a range of chemical recalcitrance and nutrient content. The substrates were prepared to deliver 30 mg g^{-1}C to the soil.

After the soil incubation, 25 μl of substrate was dispensed into each well of the deep well plate containing the soil. Subsequently, the plates were left open for 30 min to allow the release of CO_2 from carbonates present in the soil induced by the addition of acid substrates (Creamer et al., 2016). Despite this, the respiration rate of oxalic acid had quite a strong positive relationship with the pH across our samples (partial correlation $r = 0.53$, Fig. S1), and for this reason we decided to remove it from the analysis. The initial colorimetric values of the detection plates were read at 570 nm to obtain initial absorbance values (T_0) before the deep well plates were sealed and incubated at 25 °C for 6 h. Following the incubation, the colorimetric values of the detection plates were read again (T_1) and these final absorbance values were normalized using the initial absorbance values at T_0. The absorbance data were converted to CO_2 concentration using a calibration curve: $\%CO_2 = 0.02 * A_{570}^{-3.11}$ ($R^2 = 0.93$), where $\%CO_2$ is the concentration in the headspace after incubation and A_{570} is the normalized absorbance (Brolsma et al., 2015). The $\%CO_2$ concentrations were then converted to respiration rates (μg CO_2-C g^{-1} dry soil h^{-1}) using the formula provided in the MicroResp™ technical manual (Cameron, 2007). Thereafter, we corrected for the average respiration rate of soil to which the deionized water was added. Multiple substrate induced respiration (MSIR) was calculated as the sum of all the respired substrates per sample, and represents the total microbial functional capacity (Moscatelli et al., 2018). The absolute utilization of each substrate was converted to relative utilization by dividing it by the MSIR. This standardization removed the influence of differences in microbial biomass due to soil management (Yu et al., 2016). Shannon functional diversity index (H′) was used to assess the microbial functional diversity (Kennedy and Smith, 1995) and was determined using the formula:

$$H' = - \sum P_i \, LN \, (P_i)$$

where P_i represents the respiration induced by the ith substrate expressed as a proportion of the sum of all respiration rates. The Shannon index is used to assess the evenness of substrate utilization. As a measure of the soil basal respiration we used the standard soil respiration measured in the framework of the iSQAPER project as used for previous publications (Bongiorno et al., 2019b; Bongiorno et al., 2019c) (Table 1).

2.4. Statistical analysis

We analysed together the data from the 0–10 cm and 0–20 cm sampling depths because differences in respiration and other soil quality parameters (with the exception of POMC at $p = 0.05$) between the two soil depths were not different (Table S2) and because we used the LTEs as a random factor in all the analyses. All the statistical calculations were carried out using R version 3.6.0 and RStudio version 1.2.1335 (R Development Core Team, 2013; RStudio Team, 2016), and results were considered significant at $p \le 0.05$.

The effect of tillage, organic matter addition and their interaction on basal respiration, MSIR and H′ was assessed with linear mixed effect models (LMEs) using the function *lme* from the *nlme* package (Pinheiro et al., 2018) and was tested by analysis of variance (function *anova*). Tillage, organic matter addition and their interaction were included as fixed factors (~Tillage*Organic matter addition) while trial, block, main plot and subplot were introduced as random factors to take the nested design of the study into account (random = ~1|LTE/Block/Mainplot/Subplot) (as in Bongiorno et al., 2019b). Normality and homogeneity of variances of the residuals from the LMEs were checked both visually and with the Shapiro-Wilk and Levene's tests (Zuur, 2009). Basal respiration and MSIR were log transformed, and the H′ was elevated to power in order to meet the ANOVA assumptions.

To test the effect of the tillage and the organic matter addition on the microbial catabolic profiles we performed a permutational analysis of variance (PERMANOVA) with 10^4 permutations using Euclidean distances, using the function *adonis* from the *vegan* package (Oksanen et al., 2018). For this analysis, the absolute substrate utilization was log transformed. The LTEs were added as a random factor in the *strata* argument of the *adonis* function (Anderson, 2001). The function *betadisp* was used to perform permutational analysis of multivariate dispersion (BETADISP) with 10^4 permutations. LMEs were used to analyse the effect of soil management on the utilization of each substrate expressed as absolute and relative utilization rate, and to calculate the estimated means and the 95% confidence intervals that were used for graphical representations.

The effect of soil management on catabolic profiles was visualized with redundancy analysis (RDA) using the function *rda* in the *vegan* package with the LTEs as a conditioning factor. Statistical significance of the RDA was assessed using the *anova* function. The scores of the substrates on the first two axes of the RDA were used to assess the importance of the substrates in differentiating between soil management. Thereafter, we correlated the soil quality parameters with the first two RDA axes to check their association with the agricultural management and with the carbon substrates. The relationships between substrate utilization and environmental variables as shaped by soil management practices was visualized using RDA and tested using the *envfit* function in the package *vegan* with 10^4 permutations.

We tested the correlation between basal respiration, MSIR, H′ and substrate utilization expressed in absolute and relative utilization rate with the soil quality indicators, performing partial Pearson correlations that used the LTE as covariate, correcting for the intrinsic differences between the LTEs. For each main group of soil quality indicators (chemical, physical, biological and labile carbon fractions), we dropped indicators that had a Pearson correlation coefficient > 0.70 (Fig. S2) (total N, Ca, % silt, % sand, % clay, microbial biomass carbon and earthworm numbers). For the correlation analysis, lignin relative utilization rate and soil suppressiveness were logit transformed, sodium (Na), nematode abundance, richness and diversity were square root transformed, and the absolute substrate utilization rates and all the other soil quality parameters were log transformed to achieve linear relationships between variables. For the correlation analyses the packages *car, stats* and *ppcor* were used (Kim, 2015).

To understand which were the most important variables in explaining H′, we performed multiple linear mixed model regressions with H′ as the dependent variable and four broad groups of indicators

(i.e. chemical, physical, biological and labile organic fractions) as explanatory variables. For each starting model, we assessed and selected the significant variables using the stepwise procedure in R (function *step*, direction "both"). To avoid problems related to multi-collinearity, for each model we selected explanatory variables with *vif* not higher than 3. We then ran the multiple regression models for each group with the significant variables to assess the Akaike Information Criterion (AIC), the R_m^2 (marginal coefficient of determination), which indicates the proportion of the variation explained by the predictor variables, and the R_c^2 (conditional coefficient of determination), which indicates the variation explained by both the fixed and the random factors. In addition, we calculated the R_{mAdj}^2 to give a measure of the accuracy of the model across different samples (Field et al., 2012). The models were also used to assess the t and the *p*-values, which quantify the contribution of each predictor to the model (Field et al., 2012) and the significance, respectively, of the explanatory variables. We then used the parameters that resulted to be significant ($p \leq 0.05$) from these four regressions for a final multiple mixed model regression. To take the nested structure of the experimental design of the LTEs into account, we allowed the intercept to vary depending on the LTE (Zuur, 2009). Normality and homogeneity of variances of the residuals from the models were checked both visually and with the Shapiro-Wilk and Levene's tests (Zuur, 2009).

Finally, we performed piecewise structural equation modelling (SEM) to assess the direct and indirect effects of labile carbon and soil parameters on H′, taking into account the dependent structure of the data coming from the same LTE (Lefcheck, 2016) using the package *piecewiseSEM*. An a priori model was established according to results from Bongiorno et al. (2019b) and ecological mechanisms (Fig. S3) and was used as a framework for testing and optimization of the piecewise SEM. The data matrix was fitted using log transformed variables, with the exception of H′ which was elevated to the power of two. The evaluation of the AIC was used to estimate the robustness of the models and to select the appropriate final model (Shipley, 2013). The Fisher Chi-square test (χ^2; the model has a good fit when $0 \leq \chi^2/d.f. \leq 2$ and $p \geq 0.05$) was used to test the overall goodness of fit of the model (Lefcheck, 2016). We calculated and reported the total standardized effects of the predictors on soil functional diversity (H′). In the path diagram standardized effects sizes are reported as path coefficients close to the arrows representing relationships between variables, and the R_m^2 and the R_c^2 are reported only for the dependent variables.

3. Results

3.1. Effect of soil management on microbial respiration and catabolic profiles

The basal respiration was on average 0.38 μg CO_2-C g^{-1} soil h^{-1}, and was 51% higher in reduced tillage compared to conventional tillage (Table 2). Reduced tillage and high organic matter addition increased the multiple substrate induced respiration (MSIR) by 37% and 32%, respectively, compared to conventional tillage and low organic matter addition.

The absolute utilization rate of five out of six substrates increased in response to reduced tillage and high organic matter addition (Fig. 1A and Table S3). Only the utilization of amino-butyric acid showed a small but significant increase in response to reduced tillage and was not affected by organic matter addition. Alpha-ketoglutaric acid was the most utilized substrate in terms of absolute and relative respiration rate in all treatments (Fig. 1 and Table S3).

Relative utilization rates of the different substrates were more similar between management classes than absolute utilization rates (Fig. 1B). Nevertheless, the relative utilization rate of the substrates increased in response to reduced tillage compared to conventional tillage, except for amino-butyric acid and lignin, which were not affected by tillage, and for alpha-ketoglutaric acid, which was enhanced by

conventional compared to reduced tillage (Fig. 1B and Table S3). Lignin was the only substrate whose utilization was enhanced by the high compared to the low organic matter addition treatment. As a result, the relative substrate use in reduced tillage treatments with low and high organic matter addition was more similar and even than in the conventional tillage treatments with low and high organic matter addition, in particular the conventional tillage with low organic matter addition.

For both absolute and relative substrate utilization rate, PERMANOVA showed that the conditional variable (LTE) explained approximately 80% of the variation (Table S4). Nevertheless, the PERMANOVA for the absolute substrate utilization rate also showed an effect of both tillage ($p = 0.002$) and organic matter addition ($p = 0.003$) (Table S4). In the RDA plot of the substrate utilization, expressed as absolute utilization rate, the two most extreme management practices (CT-Low and RT-High) are separated along axis 1, and the two intermediate management practices (CT-High and RT-Low) are both situated in the middle, closer to RT-High than to CT-Low (Fig. 2A). All the substrates were positively correlated with RDA axis 1 (average correlation 0.89, Table S5), indicating that they were all more utilized in conventional tillage with high organic matter, in reduced tillage with low organic matter and, most of all, in reduced tillage with high organic matter addition (Fig. 2A).

All the carbon substrates, except alpha-ketoglutaric acid, were equally important (the score on the RDA axis 1 was 0.24 for alpha-ketoglutaric acid and about 0.50 for all the other substrates) in explaining the differences in the catabolic profiles expressed as absolute utilization rate between the different treatments on RDA axis 1 (Table S5). None of the substrates had a high score on RDA axis 2.

Regarding the utilization of the substrates expressed as relative utilization rate, PERMANOVA showed significant main effects of tillage ($p = 0.006$) and organic matter addition ($p = 0.03$), and a significant interaction between tillage and organic matter addition ($p = 0.04$) (Table S4). PERMANOVA results are visualized in the RDA plot, which shows that the catabolic profile in conventional tillage differed from that in reduced tillage, regardless of the organic matter addition level (Fig. 2B). Organic matter addition only had an effect on the utilization profiles in conventional tillage, which explains the interaction between tillage and organic matter addition found in the PERMANOVA. Moreover, the most intensive soil management combination (CT-Low) was clearly separated from the others on RDA axis 1, similarly to the RDA plot of the absolute utilization rate (Fig. 2A). The position of alpha-ketoglutaric was orientated in the direction of CT-Low, (score = 0.24 and correlation coefficient $r = -0.99$ with $p < 0.001$ on the RDA axis 1, Table S5).

3.2. Effect of soil management on microbial functional diversity

The Shannon functional diversity index (H′) showed a small but significant increase (6%) in reduced tillage compared to conventional tillage, and it was not significantly affected by organic matter addition (Table 2). The contribution of alpha-ketoglutaric acid in diminishing the functional diversity in conventional tillage, especially with low OM addition, is evident in Fig. 1B. The utilization of alpha-ketogularic acid expressed in absolute utilization rate was not correlated with H′ ($r = 0.13$), and the one expressed in relative utilization rate was negatively correlated with H′ ($r = -0.89$, $p < 0.001$) (Table S6).

3.3. Relationships between catabolic profiles and soil properties

The MSIR was positively associated with various soil parameters, and these relationships well reflected relationship between soil parameters and absolute utilization rates of all the substrates, in particular lignin, alanine, *N*-acetyl glucosamine, and glucose utilization (Table 3, Table S7). Several soil parameters were positively correlated with RDA axis 1 of the absolute respiration rates (Fig. 2A) and therefore, with less intensive soil management combinations, in particular reduced tillage

Table 2
Basal respiration, multiple substrate induced respiration (MSIR) and Shannon functional diversity index (H′), as affected by tillage (T) and organic matter addition (OM) across 10 long-term field experiments (LTEs), analysed with linear mixed effect models (total number of observations = 101). Least square means, confidence intervals (in brackets) and F and *p* values are reported for each combination of tillage and organic matter addition. Significant differences ($p \leq 0.05$) are given in bold.

	CT[a]- Low[c] (*n* = 33)	RT[b]- Low (*n* = 17)	CT- High[d] (*n* = 35)	RT- High (*n* = 16)		T	OM	T X OM	L	L/B	L/B/M	L/B/M/ S[g]
										St. dev.[i] of random effects		
Basal respiration ($\mu g\ CO_2$-C g^{-1} soil h^{-1})					Estimate	0.47	0.09	−0.13				
					St. error[h]	0.15	0.10	0.11				
					df	31	31	31				
	0.30	0.45	0.31	0.47	F	24.93	0.81	1.34				
	(0.21–0.41)	(0.32–0.64)	(0.23–0.44)	(0.33–0.67)	*p*	**< 0.001**	0.37	0.25	0.42	0.003	0.31	0.20
MSIR[e] ($\mu g\ CO_2$-C g^{-1} soil h^{-1})					Estimate	0.40	0.33	−0.17				
					St. error	0.12	0.09	0.15				
					df	31	31	31				
	4.3	5.9	5.7	7.8	F	11.94	13.62	1.37				
	(1.9–9.7)	(2.6–13.5)	(2.5–12.9)	(3.4–17.8)	*p*	**0.002**	**0.001**	0.25	1.11	0.07	0.30	0.26
H′[f]					Estimate	0.28	0.16	−0.11				
					St. error	0.18	0.10	0.16				
					df	31	31	31				
	1.27	1.35	1.32	1.40	F	7.03	2.13	0.51				
	(1.10–1.41)	(1.19–1.50)	(1.15–1.46)	(1.24–1.54)	*p*	**0.01**	0.15	0.48	0.52	0.005	0.006	0.36

[a] *CT,* conventional tillage.
[b] *RT,* reduced tillage.
[c] *Low,* low organic matter input.
[d] *High,* high organic matter input.
[e] *MSIR,* multiple substrate induced respiration.
[f] *H′,* Shannon functional diversity index.
[g] L, LTE; L/B, block nested in LTE; L/B/M, main-plot nested in block nested in LTE; L/B/M/S, subplot nested in main-plot nested in block nested in LTE.
[h] *St. error*, standard error.
[i] *St. dev*, standard deviation.

with high OM (Table 4). Total organic carbon (TOC), available Mg, basal respiration and the labile organic carbon fractions were the parameters most strongly correlated with RDA1 ($r > 0.30$). From the envfit analysis, we found that TOC, available K, basal respiration, Hy-DOC, POXC and HWEC were significantly associated with RDA axis 1 (Fig. 2A; Table S8).

The relative utilization rate of alanine and *N*-acetyl glucosamine and the Shannon functional diversity (H′) were positively correlated

with total organic carbon, P Olsen, microbial biomass nitrogen, basal respiration, and the labile carbon fractions (Table 3). In contrast, alpha-ketoglutaric acid had an opposite pattern than the other substrates, showing a negative correlation with total organic carbon, P Olsen, microbial biomass nitrogen, basal respiration, and the labile carbon fractions (Table 3). This confirmed the results of the RDA, where alpha-ketoglutaric acid was located away from all the other substrates (Fig. 2B). Total organic carbon, Olsen P, microbial biomass nitrogen
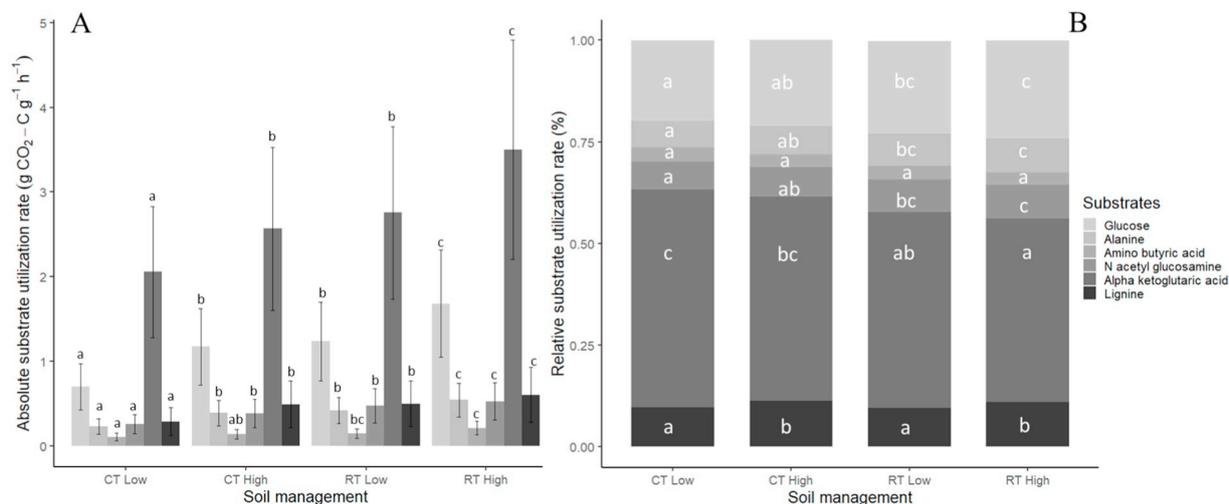
Fig. 1. Mean utilization of six substrates expressed in A) as absolute utilization rate ($\mu g\ CO_2$-C g^{-1} soil h^{-1}) and in B) as relative utilization rate (%) for each combination of tillage and organic matter addition across ten European long-term field experiments (total number of observation = 101). The error bars in A) represent 95% confidence intervals. Different letters indicate significant differences in substrate utilization per substrate between treatments (number of observations CT Low = 33, CT High = 35, RT Low = 17 and RT High = 16). *CT*, conventional tillage; *RT*, reduced tillage; *High*, high organic matter addition, *Low*, low organic matter addition.
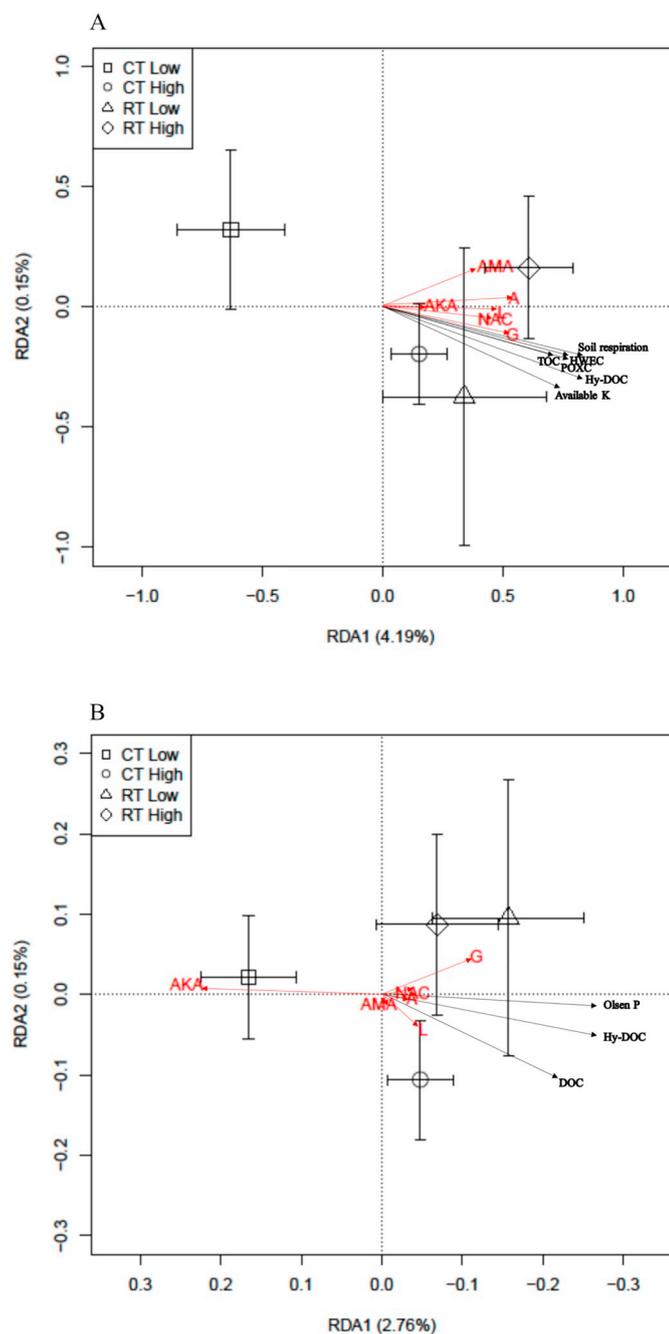
Fig. 2. Redundancy analysis (RDA) showing the effects of tillage and organic matter addition, displayed as centroids with standard error bars, on the catabolic profiles. In A) substrate utilization refers to absolute utilization rate (μg CO_2-C g^{-1} soil h^{-1}) and in B) to relative utilization rate (%). In both panels, the substrates are shown with red arrows and soil parameters are shown with black arrows (only the significant ones at $p \leq 0.05$). CT, conventional tillage; RT, reduced tillage; High, high organic matter addition; Low, low organic matter addition; G, glucose; A, alanine; NAC, N-acetyl glucosamine; AMA, aminobutyric acid; L, lignin; TOC, total organic carbon; POXC, permanganate oxidizable carbon; HWEC, hot water extractable carbon; Hy-DOC, hydrophilic dissolved organic carbon; DOC, dissolved organic carbon. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

and the labile carbon fractions had a strong positive correlation with RDA axis 1 of the relative respiration rates (r > 0.30) (Table 4). In addition, according to the envfit analysis, Olsen P, basal respiration, Hy-DOC, and DOC were positively associated with RDA axis 1

($p < 0.05$) (Fig. 2B; Table S8).

3.4. Variables explaining microbial functional diversity

POMC ($t = 5.75; p < 0.001$) and pH ($t = -3.53; p < 0.001$) were the variables that most explained the variation in microbial functional diversity (H′) using the LTE as a model co-variate (Model 5 in Table S9; the models used to arrive at this combined model can be found also in Table S9).

Based on our a priori SEM model (Fig. S3) and the results of the multiple regression analyses (Table S9), we constructed the mechanistic relationships between H′ and soil parameters using piecewise structural equation modelling (Fig. 3). POMC, positively affected by reduced tillage and organic matter addition, had a direct positive effect on H′, whereas pH had a direct negative effect on H′. POMC stimulated microbial biomass nitrogen and basal respiration, but these two microbial parameters did not significantly affect H′. The variables selected from the multiple regression explained 56% of the variation in H′ (Fig. 3).

4. Discussion

4.1. Soil management affects microbial respiration and catabolic profiles

In accordance with our first hypothesis, microbial activity and catabolic profiles were affected by the soil management tested. Reduced tillage and high organic matter addition increased the multiple substrate induced respiration (MSIR), compared to conventional tillage and low organic matter addition. The changes in catabolic profiles expressed as absolute utilization rate strongly reflected the changes in MSIR, indicating that decreasing agricultural intensity increased soil microbial activity, in accordance with the literature (Glodowska and Wozniak, 2019; Zuber and Villamil, 2016). The positive effect of reduced tillage and high organic matter addition on labile organic carbon (Bongiorno et al., 2019b), as found in our SEM, might explain the higher capacity of such systems to process organic matter, which, in turn, may increase their nutrient cycling capacity (Whitford and Ludwig Wade, 2002).

Although alternative soil management stimulated microbial activity for all the substrates expressed as absolute respiration rate, by analysing the relative utilization rate and showing the shifts in relative catabolic profiles we showed microbial preferences for the utilization of specific substrates in the different soil management treatments. Reduced tillage, but not organic matter addition, increased the relative utilization rate of glucose, alanine and N-acetyl glucosamine. Relative utilization of aminobutyric acid was not a sensitive indicator for soil management, as already found by Sradnick et al. (2013), while lignin relative utilization rate was higher in high organic matter input. Lignin and other aromatic compound concentrations can be increased by long-term organic matter addition (Chen et al., 2018; Liu et al., 2010), resulting in a microbial community more capable of degrading these compounds (Bugg et al., 2011; Bünemann et al., 2004). Alpha-ketoglutaric acid utilization when expressed as relative utilization rate showed a trend opposite to the other substrates, being more utilized in conventional compared to reduced tillage. Such opposite response of the microbial community to carboxylic acids compared to other carbon substrates was also found in other studies taking into account relative (Yu et al., 2016) and absolute utilization rate (Bending et al., 2000; Sradnick et al., 2013; Murugan et al., 2014).

4.2. Carbon substrate utilization discriminates between soil management practices

In accordance with our second hypothesis, the six selected carbon substrates differentially contributed to the discriminating ability of the catabolic profiles.

When taking into account the absolute utilization rates, all the

Table 3
Partial Pearson correlation coefficients (ρ) between substrate utilization (expressed as relative utilization rate (%)), Shannon microbial diversity index (H′), basal respiration and multiple substrate induced respiration (μg CO_2-C g^{-1} soil h^{-1}) and various soil chemical, physical and biological indicators, corrected for site (LTE) ($n = 101$).

	Glucose		Alanine		Amino-butyric acid	N-acetyl glucosamine		A-ketoglutaric acid		Lignin		Shannon index (H′)		Basal respiration		MSIR	
	(%)													(μg CO_2-C g^{-1} soil h^{-1})			
Chemical indicators[a]																	
TOC	0.24	*	0.38	**	0.03	0.37	**	−0.31	**	0.04		0.30	**	0.57	***	0.41	***
CEC	0.16		0.11		−0.11	−0.12		0.01		−0.03		0.07		0.38	***	0.27	**
C/N	−0.01		−0.13		−0.002	−0.03		0.04		−0.01		−0.06		−0.57	***	−0.27	**
pH	0.15		0.11		−0.13	−0.05		0.03		−0.38	***	−0.06		0.11		0.06	
P Olsen	0.32	**	0.29	**	0.08	0.34	**	−0.40	***	0.31	**	0.36	***	0.27	**	0.12	
Mg	0.11		0.07		−0.04	0.20		−0.15		0.24		0.14		0.27	**	0.42	***
K	0.24	*	0.27	**	−0.08	0.29	**	−0.24	*	0.20		0.26	*	0.38	***	0.32	**
Na	−0.11		0.03		−0.09	0.22	*	−0.01		0.08		0.04		−0.06		−0.07	
Physical indicators[b]																	
WSA	0.17		0.36	**	0.09	0.17		−0.25	*	−0.04		0.22	*	0.62	***	0.22	*
WHC	0.24	*	−0.01		−0.01	0.29	**	−0.17		0.12		0.15		0.02		0.15	
BD	−0.04		−0.10		−0.05	−0.05		−0.01		−0.07		−0.08		−0.07		−0.17	
Biological indicators[c]																	
MBN	0.25	*	0.31	**	0.08	0.42	***	−0.32	**	0.08		0.33	**	0.51	***	0.21	*
Basal respiration	0.24	*	0.35	**	0.03	0.37	**	−0.22	*	−0.07		0.27	**	1		0.44	***
Earthworm biomass	0.18		0.17		0.09	0.09		−0.17		0.11		0.17		0.07		0.15	
Nematode abundance	0.17		0.20		0.06	0.26	*	−0.16		−0.10		0.16		0.52	***	0.19	
Nematode richness	0.16		0.13		0.04	−0.01		−0.09		−0.05		0.06		0.25	*	0.09	
Nematode diversity	0.11		0.11		−0.05	0.06		0.06		−0.06		−0.01		0.15		0.0001	
Tea bag decomposition	−0.03		0.16		0.01	0.21	*	−0.08		0.04		−0.05		0.61	***	0.26	*
Soil suppressiveness	0.11		0.12		0.19	0.16		−0.15		0.03		0.17		0.15		0.07	
Labile carbon fractions[d]																	
Hy-DOC	0.23	*	0.33	*	−0.06	0.44	**	−0.37	**	0.27	*	0.33	*	0.46	***	0.28	**
Hy SUVA	−0.08		−0.01		0.09	−0.03		0.02		0.20		0.04		−0.10		−0.03	
DOC	0.14		0.36	***	0.06	0.35	***	−0.36	***	0.34	***	0.35	**	0.40	***	0.16	
DOC SUVA	0.10		−0.01		−0.01	−0.14		−0.01		0.05		0.05		−0.17		−0.02	
POXC	0.31	**	0.44	***	0.06	0.49	***	−0.40	***	0.12		0.40	***	0.76	***	0.50	***
HWEC	0.34	***	0.32	*	0.03	0.41	**	−0.35	***	0.14		0.35	*	0.63	***	0.38	***
POMC	0.28	**	0.35	**	0.006	0.43	***	−0.33	**	0.03		0.29	*	0.66	***	0.36	***

[a] *TOC* total organic carbon, *TON* total nitrogen, *CEC* cation exchange capacity, *C/N* carbon to nitrogen ratio.
[b] *WSA* water stable aggregates, *WHC* water holding capacity, *BD* bulk density.
[c] *MBC* microbial biomass carbon, *MBN* microbial biomass nitrogen.
[d] *Hy-DOC* hydrophilic carbon, *Hy SUVA* specific ultraviolet absorbance of hydrophylic carbon, *DOC* dissolved organic carbon, *DOC SUVA* specific ultraviolet absorbance of dissolved organic carbon, *POXC* permanganate oxidizable carbon, *HWEC* hot water extractable carbon, *POMC* particulate organic matter carbon.

* $p \leq 0.05$
** $p \leq 0.01$
*** $p \leq 0.001$

substrates contributed to differentiating between the soil management treatments, but particularly discriminant were the simple sugar (glucose) and the amino acids (alanine, gamma-amino butyric acid). Similarly, Romaniuk et al. (2011) and Sradnick et al. (2013) found that sugars and amino-acids contributed the most to the discrimination between different management practices in arable fields.

When expressed as relative utilization rate, the microbial response to alpha-ketoglutaric acid strongly contrasted with that to the other carbon substrates, as already mentioned. Organic acids represent one of the key metabolites present in root exudates, and they are released in the soil by plants and microbes in stressed environments for nutrient acquisition, toxicity defence and attraction of beneficial organisms (Canarini et al., 2019; Jones, 1998), whereas they are less associated with the decomposition of organic matter (Schutter et al., 2001; Sharma et al., 1998). Higher utilization of carboxylic acids with increasing level of agricultural intensity in arable systems could indicate environments where microbial communities are better adapted to degrade labile substrates (e.g. organic acids) from the rhizosphere than from plant residues, and are characterised by high reproduction rates and fast metabolism (i.e. *r*-strategists) (Romaniuk et al., 2011). According with this hypothesis, Bongiorno et al. (2019a) found that conventional

tillage had a higher enrichment index (EI), a nematode food web indicator of higher substrate availability, compared to reduced tillage. In addition, in our study it can be expected that under conventional tillage management with low organic matter addition the main organic compounds are derived from root exudates and microbial residues. On the other hand, in less intensive systems microbes might utilize more amino acids and amines, because of the larger input of such compounds with the addition of organic matter and the higher demand for nitrogen due to the higher C to N ratio of crop residues and organic matter added (Lagomarsino et al., 2012). Our study, taking into account so many different field sites, supports and strengthens the evidence presented in previous studies that showed higher utilization of carboxylic acids in more intensive soil ecosystems, i.e. arable fields compared to forest and grassland (Creamer et al., 2016; Rutgers et al., 2016), and higher utilization of amino acids, amines and carbohydrates (relative to carboxylic acids) in conservation agriculture, i.e. systems with increased crop rotation, use of cover crops and mulching (D'Acunto et al., 2018; Huang et al., 2008; Schutter et al., 2001). We therefore suggest that the utilization of carboxylic acids (e.g. alpha-ketoglutaric acid) relative to other substrates could be used as an indicator for stress in agricultural systems. However, to better value the usefulness of carboxylic acid as

Table 4
Partial Pearson correlation coefficients (ρ) between the first two RDA axes of the catabolic profiles expressed as absolute and relative utilization rate and the chemical, physical and biological parameters measured (n = 101).

	Absolute utilization rate				Relative utilization rate			
	(μg CO_2-C g^{-1} soil h^{-1})				(%)			
	RDA1		RDA2		RDA1		RDA2	
Chemical indicators[a]								
TOC	0.42	***	−0.02		0.31	**	−0.11	
CEC	0.22	*	−0.15		0.08		0.06	
C/N	−0.22	*	−0.01		0.002		0.05	
pH	0.003		−0.10		−0.02		0.10	
P Olsen	0.24	*	0.01		0.40	***	−0.08	
Mg	0.38	***	−0.13		0.15		0.03	
K	0.33	**	−0.12		0.25	*	0.005	
Na	−0.05		−0.11		0.09		0.05	
Physical indicators[b]								
WSA	0.25	*	0.09		0.24	*	−0.17	
WHC	0.17		−0.20	*	0.18		0.11	
BD	−0.16		−0.02		0.004		0.007	
Biological indicators[c]								
MBN	0.29	**	0.02		0.32	**	0.01	
Basal respiration	0.42	***	0.01		0.23	*	0.18	
Earthworm biomass	0.18		0.09		0.17		0.06	
Nematode abundance	0.20		0.06		0.17		0.09	
Nematode richness	0.08		0.04		0.12		0.002	
Nematode diversity	0.002		−0.06		−0.05		0.20	*
Tea bag decomposition	0.24	*	0.07		−0.07		−0.09	
Soil suppressiveness	0.09		0.16		0.15		−0.15	
Labile organic carbon[d]								
Hy-DOC	0.34	***	−0.09		0.37	***	−0.16	
Hy SUVA	−0.02		0.07		0.005		−0.17	
DOC	0.29	**	0.02		0.35	***	−0.27	*
DOC SUVA	−0.002		−0.06		0.07		0.06	
POXC	0.53	***	−0.001		0.40	***	0.005	
HWEC	0.44	***	−0.01		0.36	***	0.07	
POMC	0.37	***	−0.002		0.33	***	0.04	

[a] *TOC* total organic carbon, *TN* total nitrogen, *CEC* cation exchange capacity, *C/N* carbon to nitrogen ratio.
[b] *WSA* water stable aggregates, *WHC* water holding capacity, *BD* bulk density.
[c] *MBC* microbial biomass carbon, *MBN* microbial biomass nitrogen.
[d] *Hy-DOC* hydrophilic dissolved organic carbon, *Hy SUVA* specific ultraviolet absorbance of dissolved organic hydrophilic carbon, *DOC* dissolved organic carbon, *DOC SUVA* specific ultraviolet absorbance of dissolved organic carbon, *POXC* permanganate oxidizable carbon, *HWEC* hot water extractable carbon, *POMC* particulate organic matter carbon.
* $p \leq 0.05$.
** $p \leq 0.01$.
*** $p \leq 0.001$

an indicator for soil under stress, the mechanisms behind the differences between carboxylic acid utilization and the utilization of other substrates requires further study.

4.3. Reduced tillage, but not organic matter addition, increases microbial functional diversity

In accordance with our third hypothesis, and with the observed shifts in catabolic profiles due to management, reduced tillage increased slightly but significantly the microbial functional diversity (H′) compared to conventional tillage. This result is in line with findings of Lupwayi et al. (1998), Mijangos et al. (2006), Legrand et al. (2018) and Hao et al. (2019). Tillage can reduce microbial biomass and activity through direct effects (e.g. destruction of fungal mycelium) and destruction of specific microsites with higher fungal and bacterial activity (e.g. macroaggregates) (Gupta and Germida, 2015). Since macroaggregates are normally dominated by fungi, conventional tillage practices can have negative effects in particular on fungi and, therefore, on the capacity of a soil to degrade recalcitrant organic compounds (Frey, 2005). Moreover, the increased concentration of total and labile organic fractions in reduced tillage systems can serve as an additional

energy source for microbial activity (Bongiorno et al., 2019b).

In contrast with our third hypothesis, long-term organic matter addition did not significantly affect H′, in line with other studies that did not find an effect of organic addition on functional diversity (Calbrix et al., 2007; Tao et al., 2015; Zhu et al., 2017). In our case, a lack of effect might be due to the heterogeneous nature of the organic matter applied to the different LTEs, which could, depending on their chemical compositions, differ in their suitability to sustain microbial functional diversity and microbial processes such as organic matter decomposition and soil disease suppressiveness (Bongiorno et al., 2019c). Our results are in accordance with Bongiorno et al. (2019a) who did not observe an effect of organic matter input on the nematode-based food web indices structure index (SI), a measure of the degree of trophic links and capacity to recover from stress, and enrichment index (EI). In addition, it should be taken into account that nitrogen fertilization might be a confounding factor in the organic matter treatment.

Even though present, the effect of soil management on functional diversity was not very strong. However, the promotion of decomposition of various organic carbon substrates in absolute terms by reduced tillage and high organic matter addition, and the subsequent enhancement of the capacity of the soil systems to cycling nutrients
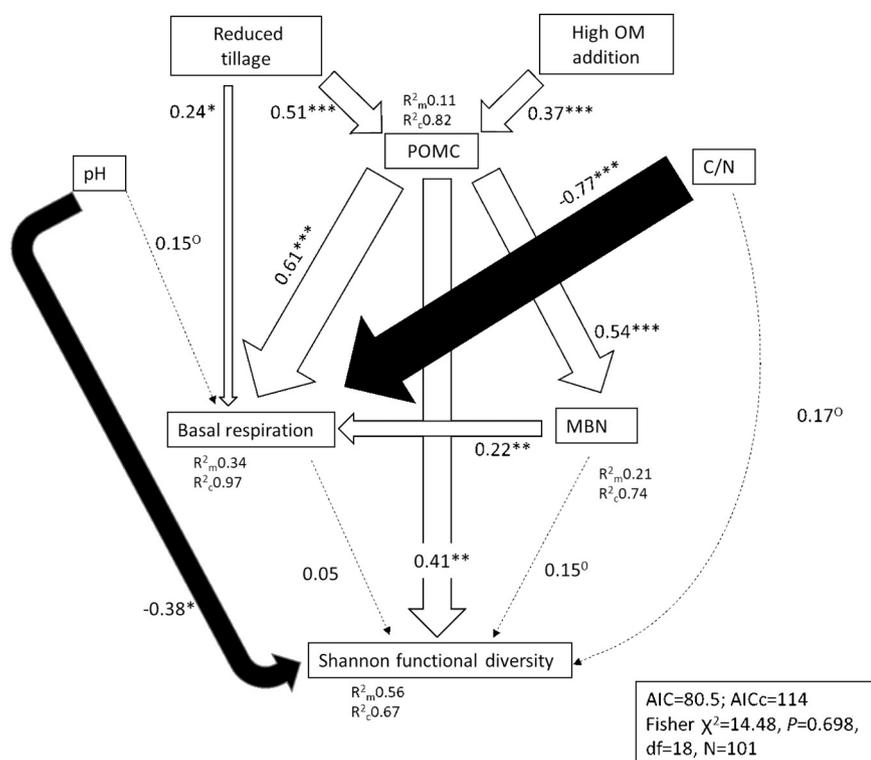
Fig. 3. Piecewise structural equation model (SEM) of soil quality parameters as predictor of Shannon functional diversity (H′). Boxes represent measured variables and arrows represent the unidirectional relationships between the parameters. Numbers on the side of the arrows indicate the standardized effect size, whose strength is proportional to the width of the arrow. White, black and dashed arrows indicate positive, negative and not statistically significant relationships, respectively. Akaike Information Criterion (AIC), corrected Akaike Information Criterion (AIC_C), Fisher chi-square (Fisher χ^2), p value (P) of the test, degrees of freedom (df), and the number of observations (N) are indicated. R_m^2 marginal coefficient of determination, R_c^2 conditional coefficient of determination, POMC particulate organic matter carbon, MBN microbial biomass nitrogen.
^{0} $p \leq 0.1$, * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$.

through organism activity, support their adoption in replacing more intensive management.

4.4. Carbon substrate utilization profiles, functional diversity and soil properties

In accordance with our fourth hypothesis, total carbon, the labile organic carbon fractions (Hy-DOC, DOC, POXC, HWEC, POMC) and parameters related to total and labile organic carbon such as macronutrients (N, P, K) and microbial characteristics (basal respiration and microbial biomass N) were strongly correlated with the catabolic profiles and microbial functional diversity (H′). These soil parameters were highest in the less intensive soil management systems. This suggests that the capacity of the microbial communities to decompose different carbon substrates was largely linked to, and probably affected by, the positive influence of conservation agriculture practices on these major soil properties.

The relationship between labile carbon as affected by soil management and the microbial community was demonstrated in our structural equation model (Fig. 3). In line with our a priori model, labile carbon expressed as POMC directly increased microbial functional diversity, but not through its positive effect on basal respiration or microbial biomass. Higher levels of available organic carbon increase the level of food availability for microorganisms and can promote microbial taxonomic and functional diversity (Huang et al., 2008; Murugan et al., 2014;; Tian et al., 2015; Yu et al., 2016; Ramirez et al., 2020). Microbial community composition and diversity, in turn, have been previously shown to correlate with organic matter decomposition (Bonner et al., 2018; Juarez et al., 2013; Maron et al., 2018).

Besides the strong direct effect of labile carbon on microbial functional diversity, also an effect of the soil pH was found (Fig. 3). pH is considered a primary driver of microbial taxonomic and functional diversity (Creamer et al., 2016; Delgado-Baquerizo et al., 2017; Moscatelli et al., 2018; Wakelin et al., 2008; Fierer and Jackson, 2006), usually positively linked to microbial activity and functionality (D'Acunto et al., 2018; van der Bom et al., 2018). Contrary to our expectation, pH had a negative direct effect on functional diversity, a

finding also reported by Zhu et al. (2017). Higher pH could have changed microbial composition, having a favourable effect on bacteria at the expenses of fungi or more oligotrophic bacteria, therefore decreasing the fungi to bacteria ratio and microbial evenness, eventually resulting in a lower microbial functional diversity. This hypothesis is supported by the negative relationship between relative utilization rate of lignin and pH, however further studies should investigate this relationship more in depth.

In summary, increasing the availability of labile carbon sources by management practices appears to be an important requirement for sustaining microbial activity and functionality, and fostering stable microbial decomposition (Bending et al., 2002; Bucher and Lanyon, 2005; Degens et al., 2000). Microbial decomposition is a required step prior to microbial assimilation of organic matter and subsequent stabilization in organo-mineral complexes (Cotrufo et al., 2013; Degens, 1998; Schmidt et al., 2011). This means that organic matter losses and the resulting reduction of functional diversity could develop in a reduced capacity of the microbial community to decompose organic matter but also to sequester carbon. Future studies should be focused on clarifying possible trade-offs between organic matter decomposition and storage caused by microbial activity in agricultural soil systems (Wood et al., 2015a).

4.5. The microbial catabolic profiles as soil quality indicator

Most of the variation in catabolic profiles was caused by the pedo-climatic zone and, possibly, the management treatments that were quite different between LTEs. Differences between specific management could not be included in the analysis (e.g. no-tillage vs. conventional tillage and non-inversion tillage vs. conventional tillage), which may have added extra variation to the results obtained (e.g. mineral N is a confounding factor in some OM treatments).

MicroResp™ uses a 'whole soil' approach and a short incubation time (6 h), trying as much as possible to approach in situ conditions (Campbell et al., 2003; Chapman et al., 2007). In addition to this, we tried to minimize possible effects of soil disturbance maintaining the differences in WHC between sites and pre-incubating the soil at 25 °C

for one week before the assay. The last is a standard step in the MicroResp™ protocol and it should ensure the activation and the equilibration of the microbial community for the assay. However, we cannot exclude that the microbial community, which was sampled in spring, experienced a stress for being exposed to such high temperature compared to the natural soil temperature. Moreover, for different sites, the best temperature for the incubation could vary. These might have had an influence on the result of the assay and it should be further taken into account when the MicroResp™ method is used across a range of different countries. That, nevertheless, the MicroResp™ system revealed effects of long-term soil management on *potential* soil microbial decomposition processes, can be considered a robust result.

One of the main challenges that we foresee in relation to the use of catabolic profiles for soil quality assessments is the establishment of optimal ranges, given that microbial spatial and temporal dynamics can be very strong, constraining the interpretation and, ultimately, the application by farmers and other land managers (Lemanceau et al., 2014; Muñoz-Rojas, 2018; Samaritani et al., 2017).

5. Conclusion

Summarizing the results from 10 long-term European arable field experiments (LTEs), we showed that MicroResp™ distinguished the activity and the functional capacity of the soil microbial community between different long-term agricultural management practices, despite the large differences in climate conditions, soil properties and specific agricultural management implementation between the LTEs. The adoption of reduced tillage and higher organic matter application were found to be effective measures for increasing the capacity of the soil microbial community to decompose various carbon substrates. Conventional tillage decreased microbial functional diversity compared to reduced tillage, as associated with higher relative utilization of alpha-ketoglutaric acid and with lower soil quality as measured by common soil parameters, in particular labile organic carbon fractions. The latter were found to play a key role in determining microbial functional diversity, probably related to food availability for microorganisms and affecting their community structure and diversity.

This makes the microbial catabolic profile a promising biological soil quality indicator. To make use of its full potential, establishment of optimal ranges for substrate utilization and microbial functional diversity measured with MicroResp™ is needed to improve the interpretation of the results and, hence, its application in monitoring at various scales (field, landscape, national) in different systems (arable, grassland, forest).

Declaration of competing interest

The authors declare that no competing interests exist.

Acknowledgements

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2020.103596.

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