

## RESEARCH ARTICLE

# Weed management modifies functional properties of both weeds and microbial nitrogen-cycling communities in Mediterranean vineyards

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**Abstract**

1. Nitrogen (N) availability is crucial to maintaining crop productivity in agroecosystems, driven primarily by soil microbial processes such as nitrification and denitrification. Weeds are an integral part of agroecosystems and are involved in many processes related to the N cycle, but how weed management could shift plant-microbe interactions, and thus, N-cycling is yet to be determined.
2. Using a network of 15 Mediterranean vineyards, we quantified the effect of 5 years of different weed management practices (chemical weeding, tillage, mowing) on the above-ground and below-ground functional properties of weed communities and soil microbial N-cycling.
3. Specific root length (SRL) of the tilled and mowed weed communities were 30% and 44% lower than in the herbicide-treated weed communities.
4. Soil pH and texture were the main drivers of soil microbial activity as quantified by substrate-induced respiration (SIR), potential denitrifying enzyme activities to SIR ratio (PDEA:SIR) and potential nitrifying and denitrifying enzyme activity ratio (PNEA:PDEA).
5. SIR was also impacted by the management: Mowed weed communities had 58% higher SIR compared to herbicide-treated communities. Weed communities with high SRL were associated with soils with a higher nitrifying enzyme efficiency per unit of respired carbon.
6. *Synthesis and applications.* Overall, our findings indicate that vineyard weed management influences the potential nitrifying enzyme activities by modifying the root strategies of weed communities. This study highlights that the design of

sustainable weed management strategies should incorporate unintended effects on soil microbial communities and N-cycling.

#### KEYWORDS

denitrifying enzyme activity, nitrifying enzyme activity, root traits, soil microbial activities, substrate-induced respiration, trait-based approach, weed communities

## 1 | INTRODUCTION

Plant–microbe interactions greatly impact soil nitrogen (N) availability in agroecosystems (Drinkwater & Snapp, 2007; Henneron et al., 2020). In addition to mineralization of organic forms of N, two major microbial processes drive plant-available N: nitrification and denitrification. Plant communities can significantly influence both nitrifier and denitrifier communities through different mechanisms, including depletion of mineral nutrients (Cantarel et al., 2015; Grigulis et al., 2013; Nuñez et al., 2022), soil moisture (Abalos et al., 2019) or the release of root exudates used as a carbon source by soil microorganisms (Li et al., 2021; Moreau et al., 2019).

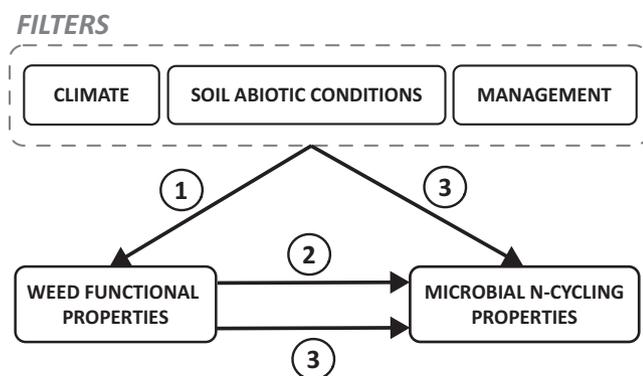
Weed communities support many agroecosystem processes like soil stabilisation (García et al., 2019) and water replenishment (García et al., 2020) but how they influence soil N cycle is yet to be determined. Perennial cropping systems, such as vineyards, present an interesting opportunity to investigate the influence of weeds on soil N-cycling because weed communities are less intensively managed and more abundant than in annual cropping systems (Meiss et al., 2010). The main weed management strategies in vineyards are tillage, chemical weed control or mowing or a combination of these three (Fernández-Mena et al., 2021). Therefore, it is essential to explore the unintended effects of these weed management strategies on soil nutrient cycling directly and indirectly through changes in weed communities.

Trait-based approaches offer a useful framework to better understand the interactions between plant and microbial communities that drive soil N-cycling (Abalos et al., 2019; Bardgett et al., 2014; Henneron et al., 2020). For plants, there is a fundamental trade-off between the rapid acquisition of resources and the efficient conservation of resources (Wright et al., 2004). These resource-use strategies are reflected in above-ground characteristics such as specific leaf area or leaf N content (Reich, 2014) and by below-ground traits such as root N content or root tissue density (Bergmann et al., 2020). Recently, Bergmann et al. (2020) discovered an additional trade-off below-ground based on interactions between plants and mycorrhizal fungi, which they termed the fungal collaboration gradient and is orthogonal to the conservation gradient. On the one side of the fungal gradient, species follow a ‘do-it-yourself’ strategy relying on soil exploration by their extensive root system (high specific root length, SRL). On the other side of the gradient, plants rely on ‘outsourcing’ to fungal partners for their acquisition of nutrients, which is characterised by a wide root diameter to provide habitat to arbuscular mycorrhizal fungi (AMF).

Trait-based studies have demonstrated that linking nitrification and denitrification by soil microbes to plant functional properties is not straightforward, showing few (Cantarel et al., 2015) or several and strong (Laughlin, 2011) relations with above-ground plant traits, as well as no link (Orwin et al., 2010), or several and strong links (Cantarel et al., 2015) with root traits. Acquisitive plant species have been associated with soils with higher nitrification potential (Cantarel et al., 2015; Laughlin, 2011; Orwin et al., 2010), explained by faster N-cycling and reduced N immobilisation in microbial biomass compared to conservative plants (Henneron et al., 2020). Some studies demonstrated that conservative species had negative impacts on denitrification potential (Abalos et al., 2019), but few studies also found positive effects (Cantarel et al., 2015; Legay et al., 2020). Recently, a growing number of studies explored the links between the collaboration gradient of the root economic spectrum and nutrient cycling (Ao et al., 2023; Han et al., 2022, 2023). However, no studies have yet shown how weed communities could influence N-cycling microbial activities through their root traits.

Management practices can impact N-cycling properties of microbial communities such as potential nitrifying and denitrifying enzyme activity (PNEA; PDEA) that can be considered as *proxies* of the soil enzyme pools involved in these processes (Fromin et al., 2010) or substrate-induced respiration (SIR) that evaluates soil heterotroph microbial activities (Beare et al., 1990). For example, tillage was shown to decrease the soils' PDEA (Wang & Zou, 2020) and SIR (Andrade et al., 2002; Hashimi et al., 2020). Mowing has been shown to have no significant impact on PNEA (Robson et al., 2007; Song et al., 2022) and PDEA (Song et al., 2022) or to increase PDEA due to higher nitrate content in soils after the mowing (Robson et al., 2007; Zhang et al., 2018). Glyphosate increases SIR in the short term (Duke et al., 2012) but decreases it in the long term (Nguyen et al., 2016). Weed management can also shape weed community functional composition in vineyards (Bopp, Kazakou, et al., 2022; Hall et al., 2020; MacLaren et al., 2019), which could indirectly impact the N-cycling of the soil. However, studies on root responses to weed management are lacking. Considering that root traits play a key role in ecosystem functioning (Bardgett et al., 2014; Freschet, Roumet, et al., 2021; Laliberté, 2016) and particularly in soil N-cycling and N use efficiency in agroecosystems (Abalos et al., 2019), there is a growing need to quantify the unintended effects of intensive (chemical weeding, tillage) and extensive weed management (mowing) on nutrient cycling through their influence on weed root traits.

The objective of this study is to explore, for the first time, the effects of weed management on microbial N-cycling through changes



**FIGURE 1** Analytical framework linking agroenvironmental filters to weed and microbial N-cycling communities. First, we explored how these filters impacted weed functional properties at the plot sampling level (arrow 1). Second, we investigated how weed functional properties related to microbial N-cycling properties (arrow 2). Third, we tested the response of microbial N-cycling properties to climate, soil abiotic characteristics, management and weed functional properties (arrows 3).

in weed communities (Figure 1). We expected that climate, soil abiotic conditions and management influence N-cycling properties of microbial communities directly and indirectly through modifying weed functional properties. We hypothesized that soil abiotic conditions would be the main direct driver of microbial N-cycling properties. We expected management to have an indirect impact on microbial N-cycling properties by modifying weed traits. We hypothesized that tillage and chemical weeding would select more acquisitive weed communities than mowing and would enhance soil microbial activities and N-cycling.

## 2 | MATERIALS AND METHODS

### 2.1 | Climate and Management of the Vineyard Network

We studied 15 vineyards in Southern France (43.62505, 3.862038, 40 km radius from East to West coast) near Montpellier (Figure 2), characterised by a mean annual temperature of 14.8°C, irregular rainfall (801 mm with 38% of rainfall occurring in autumn) and a mean aridity index of 0.66 (i.e. the ratio between annual rainfall and potential evapotranspiration) (Table S1). The soil textures ranged from sand-loamy soils around Montpellier (maximum: 70% of sands) to clay-loamy soils in the Northern part of the network (maximum 34% of clay) (Table S1). Vineyards located near the coastline of the Mediterranean Sea had clay and loamy soils. The soil samples had an average of 2% organic matter content with high variability across the different vineyards (from 0.7% to 5%) (Table S1).

Information about weed management and fertilisation practices from 2015 to 2020 was collected from farmer interviews (Table S2; Section S1). After describing our project, all farmers gave their

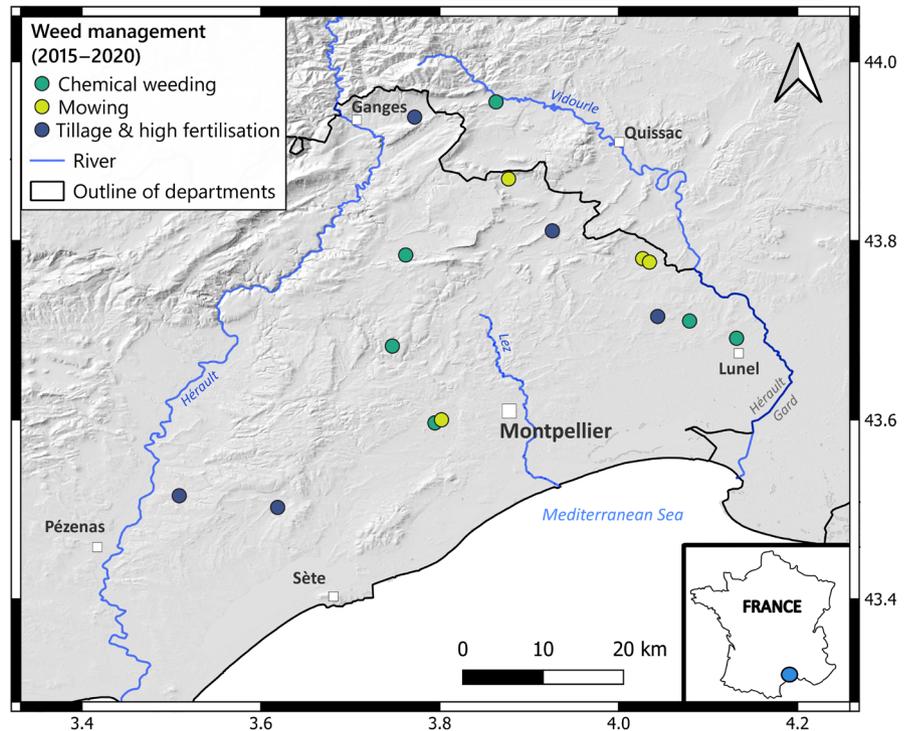
written consent for the interviews and sampling in their field. We selected the practice variables that described the level of disturbance and nutrient resource management and averaged them over 2015–2020 to consider the possible effect of management history on plant and microbial communities (Gulden et al., 2015). The frequency and the type of disturbance were described by six variables (Table S2): the frequency of chemical weeding and tillage of rows and interrows per year (interrows are the free spaces between the rows of the vines), the frequency of mowing of interrows per year and the annual longest period of weed presence between tillage or chemical weeding events. All farmers used glyphosate as an active herbicide substance. Nutrient resource management was characterised by two variables: NPK ( $\text{kg ha}^{-1}$ ) and vinasse (i.e. residuum after wort distillation) annual input (% of amended area).

### 2.2 | Above- and below-ground functional properties of weed communities

To characterise the weed communities in each vineyard, we randomly placed four quadrats ( $60 \times 60 \text{ cm}^2$ ) in neighbouring interrows (Figure 3; Figure S1) and identified weed species and their percentages of cover in March 2020 (4 quadrats  $\times$  15 vineyards = 60 sampling plots). For each sampling plot, we quantified the percentage of soil covered by vegetation and the percentage covered by legumes using visual estimation. We removed the above-ground biomass of weed communities for each quadrat, oven-dried it (60°C, 48 h) and weighed it. On the most dominant weed species (i.e. covering at least 80% of each quadrat), four above-ground traits were measured: specific leaf area (SLA), leaf dry matter content (LDMC), leaf carbon-to-nitrogen ratio (C:N) and height. SLA, LDMC and height were measured following Pérez-Harguindeguy et al. (2016) on eight distinct individuals per species collected near the quadrats in the interrows. For the leaf C and N analysis, we pooled the dried leaves of the eight individuals per plant species to obtain sufficient biomass for the analysis. The C and N content of the leaves was subsequently measured by elemental analysis (NF ISO 10694) using a FlashSmart 2000NC HPAR analyser (ThermoFisher Scientific, Mylan, Italy). To scale up from species to communities, we calculated the community-weighted means (CWM) of the leaf traits (see the equation in Section S2) (Garnier et al., 2004).

Four root traits were measured at the plant community level. Roots were extracted from one soil core (diameter 8 cm, 10 cm deep) sampled in each quadrat (Figure S1). The roots were washed with tap water and fine roots were selected (<2 mm in diameter) and scanned at 800 dots per inch (Epson Expression 1680; Epson, Ontario, Canada). The root scans were processed with digital image analysis software (WinRhizo Pro, v.2009c; Regent Instruments, Quebec, Canada). For each scanned root sample, the total root length and root volume per diameter class were determined using WinRhizo software. Then, the root samples were dried in an oven (60°C, 48 h) and weighed. The C:N of the roots (root C:N) was quantified

**FIGURE 2** Location of the 15 vineyards around Montpellier, Southern France and their management. Each dot is a vineyard whose colour refers to weed management applied by the farmers: Green, vineyards with herbicide-treated rows and interrows during 5 years; light green, vineyards with interrows with a long period of weed cover presence and inputs of vinasse during 5 years; blue, highly fertilised vineyards with tilled interrows and herbicide-treated rows during 5 years. The blue lines represent the main rivers of the region. The dark lines represent the outlines of the departments.



following the same methodology as for the leaves. The mean diameter ( $D$ ), the SRL (total root length to dry root mass ratio) and the density of the root tissue (RTD; root mass to root volume ratio) were calculated based on the scan and dry root mass analyses.

### 2.3 | Soil abiotic characteristics and potential microbial activities

To assess the abiotic characteristics of the soil in each vineyard, we sampled 10 cores per vineyard (0–10 cm top soil layer), using a soil auger (diameter 8 cm), in March 2020. The 10 cores were collected within the four neighbouring interrows near the sampling plots (Figure S1). We pooled the 10 soil samples into one soil sample per vineyard for physico-chemical analyses to obtain the average soil abiotic characteristics for each vineyard (Table S1).

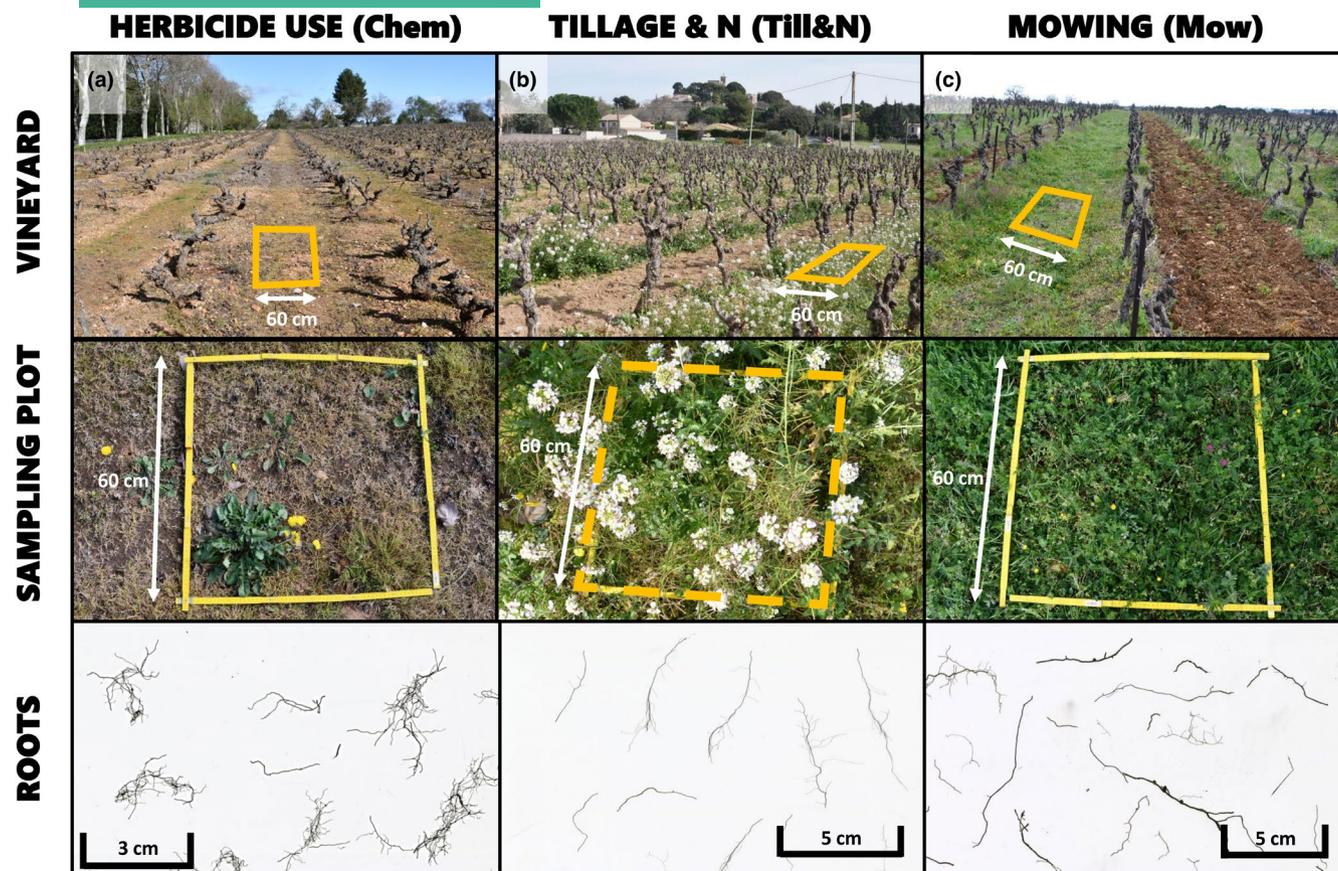
To characterise the potential microbial activities of the soil, three soil additional cores were sampled within each quadrat on the same days (diameter 8 cm, 10 cm depth), near the cores sampled to extract roots (Figure S1). Three potential microbial activities were measured: (i) SIR, (ii) nitrifying enzyme activity (PNEA) and (iii) denitrifying enzyme activity (PDEA). These variables indicate the potential activities of different enzyme pools with no limiting factors (Fromin et al., 2010). SIR was measured following Beare et al. (1990) and was expressed as the amount of C-CO<sub>2</sub> produced per gram of dry soil per hour (see more details in Section S3). PDEA was measured following Smith and Tiedje (1979) and PNEA following Lensi et al. (1986) (see more details in Section S3). Four microbial variables were used to characterise the microbial N-cycling properties. First, SIR was selected as a proxy for heterotrophic microbial activity. Second, the ratios of PDEA:SIR and

PNEA:SIR were calculated to estimate the efficiency of the enzyme pools to convert N forms per respired carbon unit. Finally, PNEA:PDEA was calculated the balance between nitrification and denitrification.

### 2.4 | Data analyses

To identify the groups of vineyards with similar weed management practices, we first performed a principal component analysis (PCA), based on Euclidean distances, on management practice variables extracted from farmer interviews from 2015 to 2020 (Figure S2A) and then a hierarchical ascendant clustering based on the PCA coordinates of the 15 vineyards (Figure S2B). Vineyard management was classified into three groups (Figure S1B; Table S2): (i) highly fertilised vineyards with tilled interrows and herbicide-treated rows ('Till&N', five vineyards), (ii) vineyards with herbicide-treated rows and interrows ('Chem', six vineyards) and (iii) vineyards with interrows with a long period of weed cover presence and vinasse inputs ('Mow', four vineyards). We checked the dependency of pedoclimatic characteristics and management groups, but no significant relations were found (Figure S3).

In the present study, two data sets were used: the plot-level data set ( $n=60$  sampling plots with their plot-level plant community) and the vineyard-level data set ( $n=15$  vineyards). To characterise the covariations between above-ground and below-ground plant traits at the community level, we calculated a PCA based on the data set at the plot level of the plant functional traits ( $n=60$  sampling plots). We then used ANOVA (type III) to test whether weed management (Till&N, Chem, Mow), climate (aridity index was selected as this index captured most of the climatic variation)



**FIGURE 3** Pictures of vineyards, weed communities (sampling plots) and their roots managed with herbicides (a), tillage & high N fertilisation rates (b) and mowing with vinasse inputs (c). Top panels show the interrows of the vineyards where one weed community was sampled, middle panels are pictures of the sampling plots, below panels are the scans of roots that were sampled within the quadrats of weed communities shown in the middle panels.

and soil parameters (pH, soil organic matter, % sand, soil C:N) significantly affected the functional properties of weed communities (CWM, community-level root traits) based on their CWM coordinates along the first three axes of the functional traits PCA and root biomass. To test whether the microbial N-cycling properties (SIR, PNEA:SIR, PDEA:SIR, PNEA:PDEA) were related to the functional properties of the weeds, we performed mixed linear models using the plot-level data set ( $n=60$ ). In each model, we added metrics of functional properties of the weeds as fixed effects and the 'vineyard identity', that is, the vineyard site where the communities were sampled, as a random effect. As the above-ground biomass and the root biomass were positively correlated ( $\rho=0.34$ ,  $p=0.01$ ), we chose to only keep the root biomass in the models. To explore the effect of management, soil, climate and weed functional properties that were significantly related to microbial N-cycling properties (SIR, PNEA:SIR, PDEA:SIR, PNEA:PDEA), we performed linear models using the vineyard-level data set ( $n=15$ ). For each model presented in this paper, we checked that the variation inflation factor was less than five. Then, we selected the best-explaining model using the corrected Akaike information criterion (AICc  $<2$ ). All data analyses were carried out using R version 4.1.1 (R Core Team, 2021).

### 3 | RESULTS

#### 3.1 | Management effects on weed traits

Management had strong effects on weed biomass, richness, composition and some weed traits. The above-ground dry biomass of the mowed weed communities ( $147\text{g m}^{-2}$ ) was significantly higher than the biomass of herbicide-treated communities ( $59\text{g m}^{-2}$ ) (Figure S4A). Also, the root biomass of the weed communities in mowed vineyards was significantly higher ( $15.6\text{g m}^{-2}$ ) than the root biomass of herbicide-treated ( $5.8\text{g m}^{-2}$ ) or tilled ( $6.6\text{g m}^{-2}$ ) vineyards (Figure S4F). The percentage of vegetation cover of the mowed communities (91%) was also significantly higher compared to the tilled and herbicide-treated communities (59%) (Figure S4B). The mowed communities counted significantly more species ( $37\text{ species m}^{-2}$ ) than the tilled and herbicide-treated communities (20 and 14  $\text{species m}^{-2}$ , respectively) (Figure S4C). The legume species were significantly more abundant in the mowed and tilled communities (representing 36% and 29% of the vegetation cover, respectively) compared to the communities under chemical weeding (0% legume cover) (Figure S4E). Perennial species were significantly more abundant in herbicide-treated vineyards (48%) than in vineyards managed with tillage (7%) (Figure S4D).

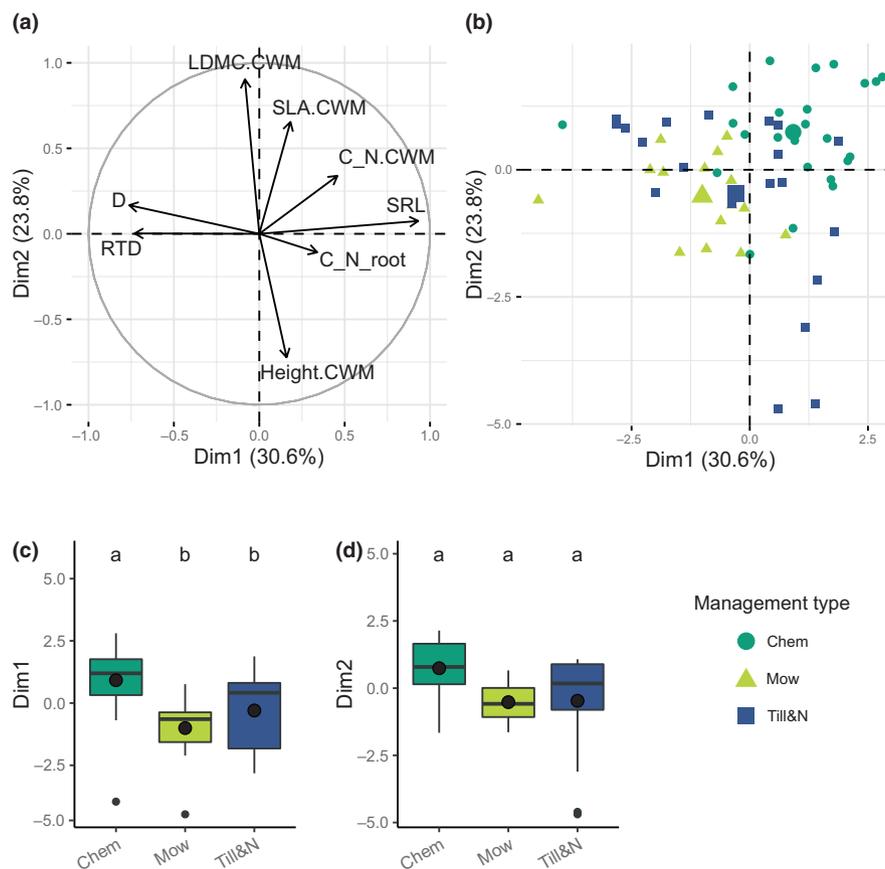
Fifty-five per cent of the functional trait variability across plant communities (Figure 4A) was explained by the first two axes of the PCA exploring above-ground and root trait covariations. The first axis was driven by root traits at the community level (30.6% of the explained variation). This axis opposed weed communities with high SRL to communities with high root diameters (D) and high RTD. SRL correlated negatively with the diameter of the roots ( $r = -0.72$ ,  $p < 0.001$ ) and the RTD ( $r = -0.71$ ,  $p < 0.001$ ) (Table S3). The second PCA axis was explained by above-ground CWM parameters (23.8% of explained variation). This axis discriminated weed communities dominated by species with high LDMC and high SLA from tall plants. SLA and LDMC were positively correlated ( $r = 0.63$ ,  $p < 0.001$ ) (Table S3). The third PCA axis was driven by SLA, height, root C:N and leaf C:N and explained 16% of the variance in the functional traits (Figure S5A). Leaf C:N was positively correlated with root C:N ( $r = 0.44$ ,  $p = 0.02$ ) and with SRL ( $r = 0.44$ ,  $p = 0.01$ ) (Table S3).

The root traits of the weed communities in the herbicide-treated vineyards contrasted with those of the tilled or mowed weed

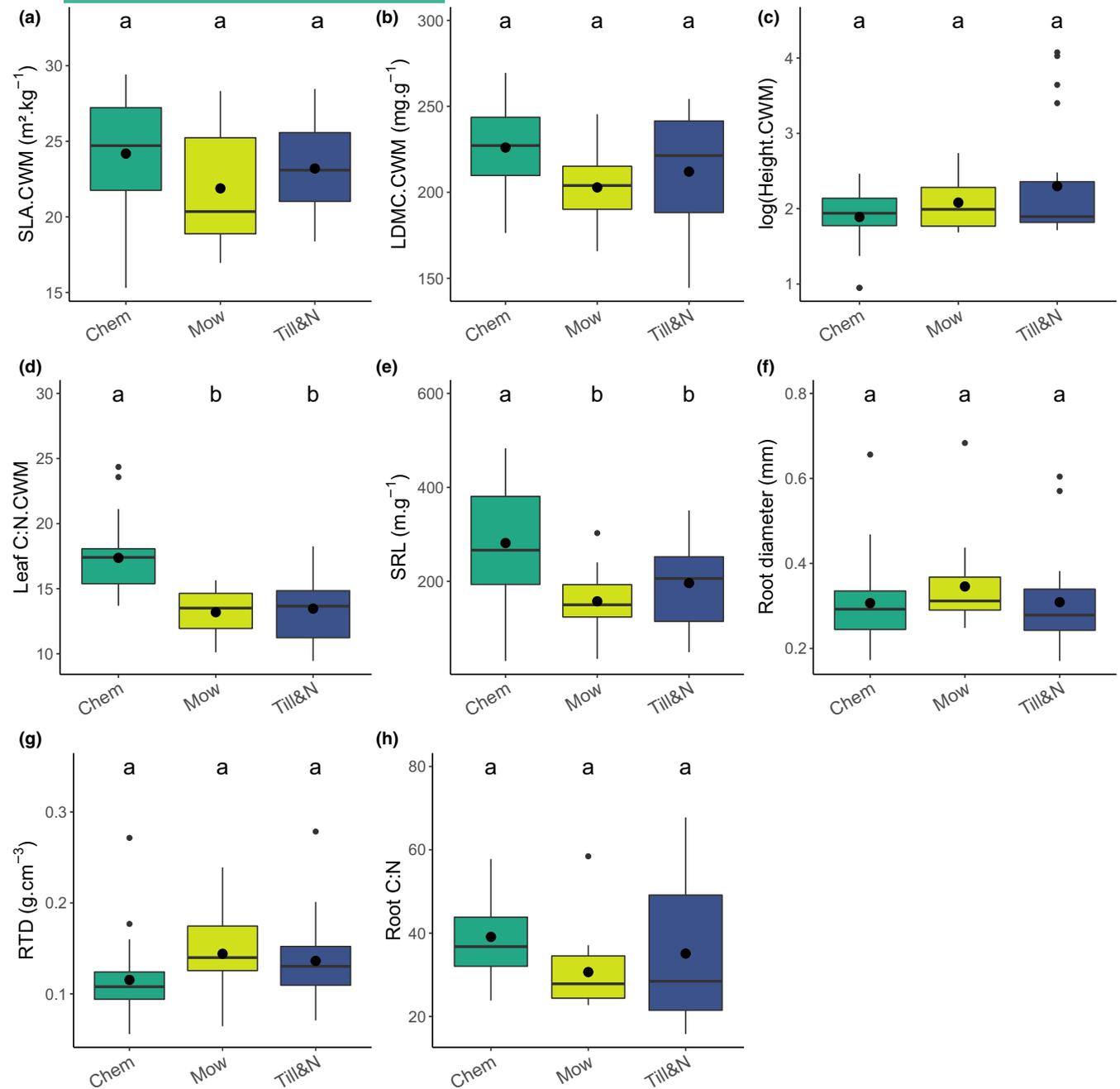
communities, as evidenced by the position of the root trait values along the first axis of the PCA functional traits (Figure 4B,C). Weed communities controlled by chemical weeding had significantly higher SRL ( $282 \text{ mg}^{-1}$ ) than weeds subjected to tillage ( $196 \text{ mg}^{-1}$ ) or mowing ( $157 \text{ mg}^{-1}$ ) (Figure 5E; Table S4). Weed communities in vineyards subjected to chemical weeding had higher CWM leaf C:N (17.4) than tilled (13.2) and mowed communities (13.5) (Figure 5D, Table S4). The weed communities in sandy soils had higher leaf C:N than the communities in silty soils (Table S4). SLA.CWM, LDMC.CWM, Height.CWM, root diameter, RTD and root C:N were not significantly different between weed management treatments (Figures 4D and 5A-C,F-H).

### 3.2 | Weed traits associated with N-cycling properties of microbial communities

Only one root trait was associated with N-cycling properties of microbial communities, while no above-ground plant traits were selected in



**FIGURE 4** (A) First two axes of a principal component analysis (PCA) based on the weed community above-ground community-weighted means (CWM) traits and community-level root traits. (B) Weed community properties as projected in trait space according to the first two axes of the functional traits PCA. Each dot is a weed community whose colour and form refer to weed management of the corresponding vineyard; the larger sized symbols indicate the centroid per treatment. (C) Boxplot of weed community coordinates along the first axis of the functional traits PCA according to weed management. (D) Boxplot of weed community coordinates along the second axis of the functional traits PCA according to weed management. C.N.CWM, leaf carbon to nitrogen ratio; C.N.root, carbon to nitrogen ratio of the roots; Chem, vineyards with herbicide-treated rows and interrows during 5 years; D, root diameter; LDMC.CWM, leaf dry matter content; Mow, vineyards with interrows with a long period of weed cover presence and inputs of vinasse during 5 years; RTD, root tissue density; SLA.CWM, specific leaf area; SRL, specific root length; Till&N, highly fertilised vineyards with tilled interrows and herbicide-treated rows during 5 years. Different letters indicate significant differences of PCA coordinates according to weed management.



**FIGURE 5** Boxplot of weed above-ground community-weighted means (CWM) traits and root traits at the community level according to the weed management practices : SLA (A), LDMC (B), Height (C), Leaf C:N (D), SRL (E), Root diameter (F), RTD (G) and Root C:N (H). Acronyms are specified in Figure 4. Different letters indicate significant differences of trait values according to weed management.

the models. Weed communities with high SRL were associated with vineyard soils with high PNEA:SIR ( $\beta$ : 0.48,  $p=0.016$ , Table 1). SIR, PDEA:SIR and PNEA:PDEA were not significantly explained by the above-ground CWM traits, the root traits or the root biomass. Most of the variances of SIR, PDEA:SIR and PNEA:PDEA were explained by the 'vineyard' random effect (78% for PDEA:SIR, 86% for SIR and 67% for PNEA:PDEA), suggesting that pedoclimatic conditions and management could strongly impact these biochemical variables of the soil. The coordinates of the weed communities in the first three dimensions of the functional trait PCA did not explain the N-cycling properties of microbial communities (Table S5).

### 3.3 | Effects of management, soil, climate and weed traits on N-cycling properties of microbial communities

N-cycling properties of microbial communities were explained primarily by abiotic conditions in the soil. Among all the variables tested, only the abiotic characteristics of the soil (pH and % sand) and the management of weeds were selected in the models explaining the variation in SIR (Table 2). Acidic soils with a low sand content had a high SIR. The SIR was highest in mowed vineyards ( $17.2 \mu\text{g C-CO}_2 \text{g}^{-1} \text{h}^{-1}$ ), lowest in tilled vineyards ( $7.1 \mu\text{g C-CO}_2 \text{g}^{-1} \text{h}^{-1}$ ) and in herbicide-treated vineyards

**TABLE 1** Standardised estimates of the weed functional properties (above-ground community-weighted means, CWM; community-level root traits and root biomass) explaining microbial N-cycling properties (SIR, PNEA:SIR, PDEA:SIR and PNEA:PDEA) ( $n=60$  sampling plots).

Explained variables	Explaining variables	$\beta$	Std. err.	$t$	$p$	$R^2_{\text{marg}}$	$R^2_{\text{cond}}$	$\text{AICc}_{\text{full}}$	$\text{AICc}_{\text{sel}}$
SIR	Root biomass	-0.04	0.03	-1.19	0.241	1%	87%	106.6	54.9
PNEA:SIR	SRL	0.48	0.19	2.50	<b>0.016</b>	12%	39%	-91.6	-319.9
	RTD	0.34	0.18	1.93	0.060				
PDEA:SIR	-	-	-	-	-	0%	78%	147.3	103.4
PNEA:PDEA	-	-	-	-	-	0%	67%	35.2	-30.5

Note: Significant  $p$ -values ( $p < 0.05$ ) are in bold. Full model: Explained variable ~ SLA + LDMC + height + leaf C:N + SRL + root diameter + RTD + root C:N + root biomass + (1|Vineyard).

Abbreviations:  $\text{AICc}_{\text{full}}$ , corrected Akaike information criterion of the full model;  $\text{AICc}_{\text{sel}}$ , AICc of the selected model;  $p$ ,  $p$ -value; PDEA, potential denitrifying enzyme activity; PNEA, potential nitrifying enzyme activity;  $R^2_{\text{cond}}$ , conditional  $R^2$ ;  $R^2_{\text{marg}}$ , marginal  $R^2$ ; RTD, root tissue density; SIR, substrate-induced respiration; SRL, specific root length; Std. err., standardised errors;  $t$ ,  $t$ -value;  $\beta$ , standardised beta estimates.

**TABLE 2** Standardised estimates of climate, soil, management and weed functional properties explaining the microbial functional properties ( $n=15$ ).

Explained variables	Explaining variables	$\beta$	Std. err.	$t$	$p$	$R^2$	$\text{AICc}_{\text{full}}$	$\text{AICc}_{\text{sel}}$
SIR	pH	-0.28	0.07	-4.07	<b>0.001</b>	74%	48.8	37.3
	% sand	-0.27	0.07	-3.13	<b>0.002</b>			
	Weed management 'Mow' compared to 'Chem'	0.36	0.16	2.36	<b>0.040</b>			
	Weed management 'Till&N' compared to 'Chem'	0.12	0.14	0.31	0.762			
PNEA:SIR	SRL	0.53	0.23	2.27	<b>0.041</b>	8%	-58.8	-99.4
PDEA:SIR	pH	0.24	0.08	4.95	<b>&lt;0.001</b>	68%	56.1	32.4
	% sand	-0.21	0.08	-2.57	<b>0.024</b>			
PNEA:PDEA	SRL	0.43	0.22	1.99	0.07	49%	24.4	-7.4
	pH	-0.68	0.22	-3.16	<b>0.008</b>			

Note: Significant  $p$ -values ( $p < 0.05$ ) are in bold. Acronyms are specified in Table 1 and Figure 2. Full model: Explained variable ~ management + pH + organic matter + % sand + aridity index + SRL.

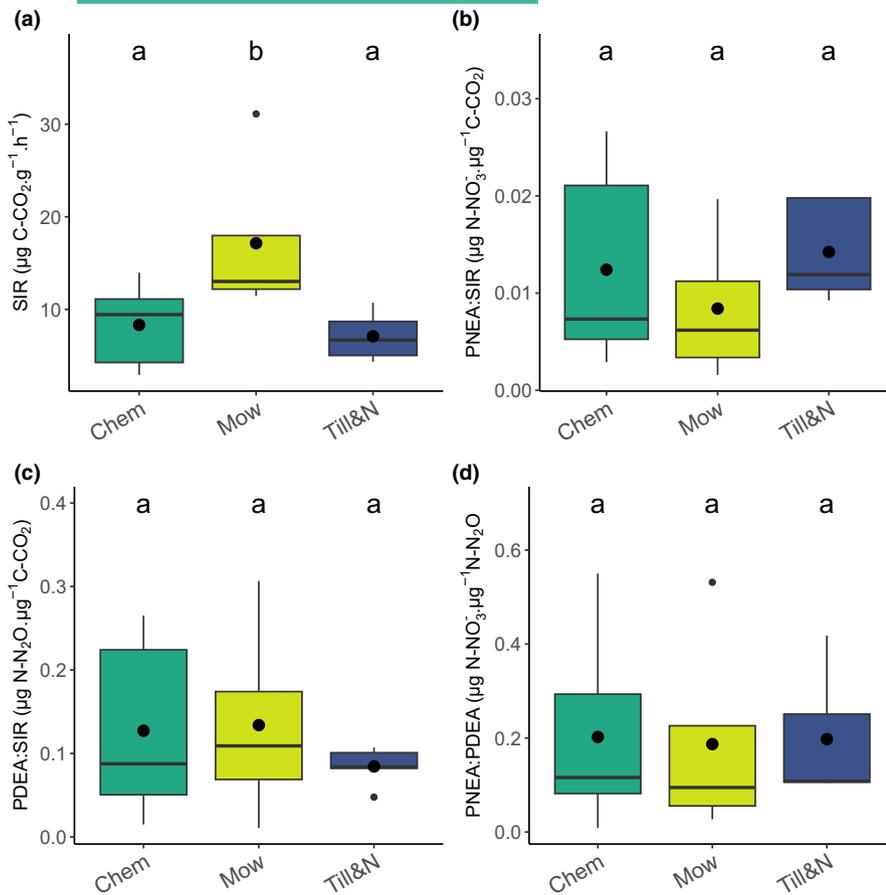
( $8.3 \mu\text{g C-CO}_2\text{g}^{-1}\text{h}^{-1}$ ) (Figure 6A). Weed communities with do-it-yourself strategies (high SRL) were associated with microbial communities with higher efficiency of nitrification per unit of respired carbon ( $\beta$ : 0.53,  $p=0.041$ ; Table 2). The PDEA:SIR ratio was best explained by pH and % sand, and alkaline soils with low sand content had a higher efficiency of denitrification per unit of respired carbon. The PNEA:PDEA ratio was negatively related to soil pH, indicating a relatively higher nitrification activity in more acidic soil. Management did not explain the PNEA:SIR, PDEA:SIR and PNEA:PDEA variabilities (Figure 6B–D).

## 4 | DISCUSSION

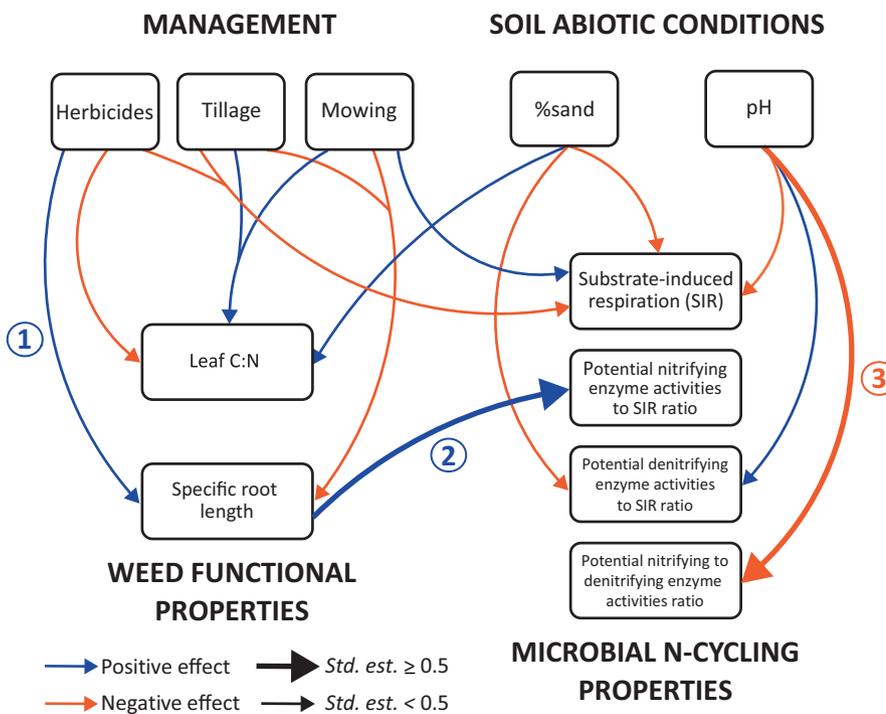
This study aimed to determine the unintended effects of weed management on N-cycling properties of soil microbial communities in vineyards through changes in weed traits (Figure 1). We found that management influenced weed traits and the activities of N-cycling enzymes in soil (Figure 7). However, soil abiotic conditions (pH and soil texture) were also important drivers.

### 4.1 | Management effects on weed above-ground and below-ground traits

We hypothesized that tillage and chemical weeding would select for more acquisitive weed communities than mowing. Five years of chemical weeding selected weed communities with long-lasting and tough leaves (23% higher leaf C:N ratio than tilled and mowed communities) and do-it-yourself strategies, based on root systems with efficient soil exploration to capture nutrients (30% and 44% higher SRL compared to tilled communities and mowed communities, respectively). In contrast to our hypotheses, herbicides selected for conservative above-ground strategies, while chemical weeding is usually conceptualised as a disturbance that would favour acquisitive plant strategies (Gaba et al., 2014). The high leaf C:N might be due to the presence of a thick cuticle or a high wax content of the leaves that decreased glyphosate absorption (Santier & Chamel, 1992). Regarding the below-ground effects, glyphosate, which was the main active ingredient in herbicides used by farmers, has been shown to



**FIGURE 6** Microbial community properties of vineyard soils according to weed management : SIR (A), PNEA:SIR (B), PDEA:SIR (C) and PNEA:PDEA (D). Acronyms are specified in Table 1 and Figure 2. Different letters indicate significant differences of microbial properties according to weed management.



**FIGURE 7** Main results of the study. First, we demonstrated that weed management was the main driver of weed-specific root length at the community level. Second, we showed that specific root length was positively related to the potential nitrifying enzyme activity (PNEA) to substrate-induced respiration (SIR) ratio (PNEA:SIR) while no functional property of weed communities correlated significantly with SIR or to the potential denitrifying enzyme activity (PDEA) to SIR ratio (PDEA:SIR). Third, we found that SIR and the PDEA to SIR ratio (PDEA:SIR) were explained by pH and the percentage of sand in the soils and PNEA:PDEA was driven by pH.

strongly decrease root colonisation by AMF and the viability of AMF spores (Druille et al., 2013). As high SRL indicates a low dependence on AMF (Bergmann et al., 2020), our results suggest that the application of glyphosate has filtered out species that collaborate with AMF.

#### 4.2 | Response of microbial activity to root traits of weeds and soil abiotic conditions

The soil microbial parameters SIR and PDEA:SIR were mainly explained by soil pH and sand content. Alkaline soils with a low sand

content had a higher PNEA:SIR. Denitrification potential was found to be negatively related to the percentage of sand in soils, likely due to better drainage and lower water-holding capacity that decrease anoxic conditions (Matson & Vitousek, 1987). Furthermore, denitrification is known to be higher in neutral or alkaline soils than in acid soils, which could be due to the indirect effect of nitrification that is reduced in acidic soils (Šimek & Cooper, 2002). This reasoning can also explain the negative relation we found between pH and the PNEA:PDEA ratio if PDEA is more sensitive to low pH than PNEA. Acidic soils with a low sand content had high SIR. In fact, fine-textured soils low in sand content have more numerous and smaller pores that physically protect microbial biomass (Hassink et al., 1993).

We hypothesized that acquisitive weed communities would favour higher soil microbial activities and faster N-cycling. SRL was the only trait that related significantly to an N-cycling microbial property of the soils. Do-it-yourself weed communities (high SRL) with a resource strategy based on extensive root systems and soil exploration were associated with a higher PNEA:SIR. Resource-acquisitive plant species (high SRL, low root diameter, low RTD) are characterised by a high N uptake (Abalos et al., 2019), high root turnover (Freschet, Pagès, et al., 2021) and a high carbon rhizodeposition (Henneron et al., 2020). This strategy has been shown to induce faster soil N-cycling due to the release of easily degradable rhizodeposits that improve N mineralization and therefore increase the soil ammonia concentration available for nitrification (Henneron et al., 2020). At the species level, with perennial species grown in pots, Cantarel et al. (2015) found that PNEA was negatively related to SRL. This contrasting result could be due to the different study scales (species vs. community), the dominance of annual weeds in the communities of our study, which occupied a different place in the root economics spectrum than perennials (Roumet et al., 2016), or the multiple environmental constraints in our in situ study that are excluded in controlled pot experiments. This highlights the need for field studies linking plant traits and soil N-cycling processes at the community level that integrate plant–plant and plant–soil microbial interactions within communities and pedoclimatic conditions.

Our study did not integrate the temporal dynamics of weeds and microbes, and therefore the dynamics of their interactions which varies over time (Hannula et al., 2019). Depending on the phenological stage, plants can affect microbial communities differently (Moreau et al., 2019). For instance, Kumar et al. (2018) showed that the roots of maize in the early development stage stimulated soil enzyme activity while this effect was reduced at maize maturity, most likely due to changes in the quality and quantity of root exudation. Incorporating a temporal dimension through various measurements throughout the seasons can provide deeper insights into how the timescale influences interactions between weeds and microbes.

#### 4.3 | Implications for the management of weeds to improve N-cycling in vineyards

There is an urgent need to limit the use of herbicides in agroecosystems and to adopt more diverse weed management practices

(Liebman et al., 2016). Achieving this change requires a deep understanding of how different practices affect agroecosystem functions, particularly soil functions. Our results suggest that herbicides indirectly increase soil nitrifying enzyme activities, by increasing SRL of the weed communities. However, we observed this as a trend and were unable to prove this statistically (e.g. using path analyses) due to our limited number of samples. However, the increased microbial nitrifying enzyme activity associated with weed communities with high SRL, favoured by herbicides, could result in increased mineral N availability for crops in the short term. Yet, at longer timescales, weed communities with high SRL could favour fast N re-mineralization (Abalos et al., 2019) and thus potentially higher N losses and lower N use efficiency (de Vries & Bardgett, 2016). In this study, we showed that mowing favoured low SRL, high RTD and high root diameter of weed communities. Therefore, mowing selected weeds with a conservative strategy and high associations with AMF, which are more likely to mitigate N losses, especially in low-fertilised agrosystems such as vineyards (Grigulis et al., 2013). This study also shows that mowing directly favoured high SIR, indicating larger soil microbial biomass, in comparison with tillage and chemical weeding. Hashimi et al. (2020) also found that no-tillage favoured higher SIR due to higher plant biomass and input of organic matter into soils (weed mulch). Our study showed that mowed weed communities produced high above-ground and below-ground biomass and were characterised by high species richness compared to weed communities managed by tillage and herbicides, also in line with the results found in orchards (Mia et al., 2020). Furthermore, using almost the same network of vineyards, Bopp, Fried, et al. (2022) demonstrated that mowed weed communities were more decomposable than herbicide-treated communities and had a higher N content within their above-ground biomass. Therefore, mowing could represent both a means to control the competition of weeds with the main crops, a way to promote interrow plant diversity and a means to serve as an amendment practice, promoting weed biomass production with high N content, favouring high soil microbial biomass and N retention in the soil.

## 5 | CONCLUSION

This study improves our understanding of the interactions between weed communities and soil N-cycling microbial communities in Mediterranean vineyards. We demonstrated the distinct effects of herbicides versus mowing on the biomass and functional traits of the weed community and the functioning of their associated soil microorganisms. Our study highlights how weed management indirectly affects soil N-cycling through its impact on the soil microbial communities and the benefits of mowing as compared to herbicides or tillage in weed management. We demonstrate the need to measure the root traits of weeds to unravel how weed communities influence soil processes and to consider the effect of the abiotic environment. Additionally, in situ studies are required to account for temporal

variations in interactions between weed and microbial communities throughout the seasons and the effect of soil abiotic conditions.

## AUTHOR CONTRIBUTIONS

Elena Kazakou, Guillaume Fried, Aurélie Metay and Marie-Charlotte Bopp conceived the first ideas and designed the experiment. Nathalie Fromin, Bruno Buatois and Florian Fort also helped to design the methodology. Gerlinde De Deyn, Marie Zwetsloot, Gabriel Y. K. Moinet and Lammert Bastiaans conceived the framework of this study. Marie-Charlotte Bopp analysed the data and led the writing of the manuscript. All authors critically contributed to drafts.

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## CONFLICT OF INTEREST STATEMENT

Elena Kazakou is an Associate Editor of *Journal of Applied Ecology*, but took no part in the peer review and decision-making processes for this paper. The authors declare no conflicts of interest.

## DATA AVAILABILITY STATEMENT

Data are available via the Dryad Digital Repository: <https://doi.org/10.5061/dryad.t4b8gtjb1> (Bopp et al., 2024).

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## REFERENCES

Abalos, D., van Groenigen, J. W., Philippot, L., Lubbers, I. M., & De Deyn, G. B. (2019). Plant trait-based approaches to improve nitrogen

cycling in agroecosystems. *Journal of Applied Ecology*, 56(11), 2454–2466. <https://doi.org/10.1111/1365-2664.13489>

- Andrade, D. S., Colozzi-Filho, A., & Giller, K. E. (2002). The soil microbial community and soil tillage. In *Soil tillage in agroecosystems* (pp. 51–81). CRC Press. <https://doi.org/10.1201/9781420040609-5>
- Ao, G., Qin, W., Wang, X., Yu, M., Feng, J., Han, M., & Zhu, B. (2023). Linking the rhizosphere effects of 12 woody species on soil microbial activities with soil and root nitrogen status. *Rhizosphere*, 28, 100809. <https://doi.org/10.1016/j.rhisph.2023.100809>
- Bardgett, R. D., Mommer, L., & De Vries, F. T. (2014). Going underground: Root traits as drivers of ecosystem processes. *Trends in Ecology & Evolution*, 29(12), 692–699. <https://doi.org/10.1016/j.tree.2014.10.006>
- Beare, M. H., Neely, C. L., Coleman, D. C., & Hargrove, W. L. (1990). A substrate-induced respiration (SIR) method for measurement of fungal and bacterial biomass on plant residues. *Soil Biology and Biochemistry*, 22(5), 585–594. [https://doi.org/10.1016/0038-0717\(90\)90002-H](https://doi.org/10.1016/0038-0717(90)90002-H)
- Bergmann, J., Weigelt, A., van der Plas, F., Laughlin, D. C., Kuyper, T. W., Guerrero-Ramirez, N., Valverde-Barrantes, O. J., Bruelheide, H., Freschet, G. T., Iversen, C. M., Kattge, J., McCormack, M. L., Meier, I. C., Rillig, M. C., Roumet, C., Semchenko, M., Sweeney, C. J., van Ruijven, J., York, L. M., & Mommer, L. (2020). The fungal collaboration gradient dominates the root economics space in plants. *Science Advances*, 6(27), eaba3756. <https://doi.org/10.1126/sciadv.aba3756>
- Bopp, M.-C., De Deyn, G., Zwetsloot, M. J., Moinet, G. Y. K., Fried, G., Metay, A., Fromin, N., Fort, F., Buatois, B., Bastiaans, L., & Kazakou, E. (2024). Data from: Weed management modifies functional properties of both weeds and microbial nitrogen-cycling communities in Mediterranean vineyards [Dataset]. *Dryad Digital Repository*. <https://doi.org/10.5061/dryad.t4b8gtjb1>
- Bopp, M.-C., Fried, G., Metay, A., Bastianelli, D., Bonnal, L., & Kazakou, E. (2022). Linkages between traits and decomposition of weed communities along a soil management and pedoclimatic gradient in Mediterranean vineyards. *Annals of Botany*, 130(4), 547–560. <https://doi.org/10.1093/aob/mcac099>
- Bopp, M.-C., Kazakou, E., Metay, A., & Fried, G. (2022). Relative importance of region, seasonality and weed management practice effects on the functional structure of weed communities in French vineyards. *Agriculture, Ecosystems & Environment*, 330, 107892. <https://doi.org/10.1016/j.agee.2022.107892>
- Cantarel, A. A. M., Pommier, T., Desclos-Theveniau, M., Diquélou, S., Dumont, M., Grassein, F., Kastl, E.-M., Grigulis, K., Laine, P., Lavorel, S., Lemauviel-Lavenant, S., Personeni, E., Schloter, M., & Poly, F. (2015). Using plant traits to explain plant–microbe relationships involved in nitrogen acquisition. *Ecology*, 96(3), 788–799. <https://doi.org/10.1890/13-2107.1>
- de Vries, F. T., & Bardgett, R. D. (2016). Plant community controls on short-term ecosystem nitrogen retention. *New Phytologist*, 210(3), 861–874. <https://doi.org/10.1111/nph.13832>
- Drinkwater, L. E., & Snapp, S. S. (2007). Nutrients in agroecosystems: Rethinking the management paradigm. In D. L. Sparks (Ed.), *Advances in agronomy* (Vol. 92, pp. 163–186). Academic Press. [https://doi.org/10.1016/S0065-2113\(04\)92003-2](https://doi.org/10.1016/S0065-2113(04)92003-2)
- Druille, M., Cabello, M. N., Omacini, M., & Golluscio, R. A. (2013). Glyphosate reduces spore viability and root colonization of arbuscular mycorrhizal fungi. *Applied Soil Ecology*, 64, 99–103. <https://doi.org/10.1016/j.apsoil.2012.10.007>
- Duke, S. O., Lydon, J., Koskinen, W. C., Moorman, T. B., Chaney, R. L., & Hammerschmidt, R. (2012). Glyphosate effects on plant mineral nutrition, crop rhizosphere microbiota, and plant disease in glyphosate-resistant crops. *Journal of Agricultural and Food Chemistry*, 60(42), 10375–10397. <https://doi.org/10.1021/jf302436u>
- Fernández-Mena, H., Frey, H., Celette, F., Garcia, L., Barkaoui, K., Hossard, L., Naulleau, A., Métral, R., Gary, C., & Metay, A. (2021).

- Spatial and temporal diversity of service plant management strategies across vineyards in the south of France. Analysis through the coverage index. *European Journal of Agronomy*, 123, 126191. <https://doi.org/10.1016/j.eja.2020.126191>
- Freschet, G. T., Pagès, L., Iversen, C. M., Comas, L. H., Rewald, B., Roumet, C., Klimešová, J., Zadworny, M., Poorter, H., Postma, J. A., Adams, T. S., Bagniewska-Zadworna, A., Bengough, A. G., Blancaflor, E. B., Brunner, I., Cornelissen, J. H. C., Garnier, E., Gessler, A., Hobbie, S. E., ... McCormack, M. L. (2021). A starting guide to root ecology: Strengthening ecological concepts and standardising root classification, sampling, processing and trait measurements. *New Phytologist*, 232(3), 973–1122. <https://doi.org/10.1111/nph.17572>
- Freschet, G. T., Roumet, C., Comas, L. H., Weemstra, M., Bengough, A. G., Rewald, B., Bardgett, R. D., De Deyn, G. B., Johnson, D., Klimešová, J., Lukac, M., McCormack, M. L., Meier, I. C., Pagès, L., Poorter, H., Prieto, I., Wurzbürger, N., Zadworny, M., Bagniewska-Zadworna, A., ... Stokes, A. (2021). Root traits as drivers of plant and ecosystem functioning: Current understanding, pitfalls and future research needs. *New Phytologist*, 232(3), 1123–1158. <https://doi.org/10.1111/nph.17072>
- Fromin, N., Pinay, G., Montuelle, B., Landais, D., Ourcival, J. M., Joffre, R., & Lensi, R. (2010). Impact of seasonal sediment desiccation and rewetting on microbial processes involved in greenhouse gas emissions. *Ecology*, 3(3), 339–348. <https://doi.org/10.1002/eco.115>
- Gaba, S., Fried, G., Kazakou, E., Chauvel, B., & Navas, M.-L. (2014). Agroecological weed control using a functional approach: A review of cropping systems diversity. *Agronomy for Sustainable Development*, 34(1), 103–119. <https://doi.org/10.1007/s13593-013-0166-5>
- Garcia, L., Damour, G., Gary, C., Follain, S., Le Bissonnais, Y., & Metay, A. (2019). Trait-based approach for agroecology: Contribution of service crop root traits to explain soil aggregate stability in vineyards. *Plant and Soil*, 435(1–2), 1–14. <https://doi.org/10.1007/s11104-018-3874-4>
- Garcia, L., Metay, A., Kazakou, E., Storkey, J., Gary, C., & Damour, G. (2020). Optimizing the choice of service crops in vineyards to achieve both runoff mitigation and water provisioning for grapevine: A trait-based approach. *Plant and Soil*, 452(1–2), 87–104. <https://doi.org/10.1007/s11104-020-04543-y>
- Garnier, E., Cortez, J., Billès, G., Navas, M. L., Roumet, C., Debussche, M., Laurent, G., Blanchard, A., Aubry, D., Bellmann, A., Neill, C., & Toussaint, J. P. (2004). Plant functional markers capture ecosystem properties during secondary succession. *Ecology*, 85(9), 2630–2637. <https://doi.org/10.1890/03-0799>
- Grigulis, K., Lavorel, S., Krainer, U., Legay, N., Baxendale, C., Dumont, M., Kastl, E., Arnoldi, C., Bardgett, R. D., Poly, F., Pommier, T., Schloter, M., Tappeiner, U., Bahn, M., & Clément, J.-C. (2013). Relative contributions of plant traits and soil microbial properties to mountain grassland ecosystem services. *Journal of Ecology*, 101(1), 47–57. <https://doi.org/10.1111/1365-2745.12014>
- Gulden, R. H., Tenuta, M., Mitchell, S., Langarica Fuentes, A., & Daniell, T. J. (2015). Preceding crop and weed management history affect denitrification and denitrifier community structure throughout the development of durum wheat. *Agriculture, Ecosystems and Environment*, 212, 49–63. <https://doi.org/10.1016/j.agee.2015.06.016>
- Hall, R. M., Penke, N., Kriechbaum, M., Kratschmer, S., Jung, V., Chollet, S., Guernion, M., Nicolai, A., Burel, F., Fertil, A., Lora, Á., Sánchez-Cuesta, R., Guzmán, G., Gómez, J., Popescu, D., Hoble, A., Bunea, C. I., Zaller, J. G., & Winter, S. (2020). Vegetation management intensity and landscape diversity alter plant species richness, functional traits and community composition across European vineyards. *Agricultural Systems*, 177, 102706. <https://doi.org/10.1016/j.agry.2019.102706>
- Han, M., Chen, Y., Li, R., Yu, M., Fu, L., Li, S., Su, J., & Zhu, B. (2022). Root phosphatase activity aligns with the collaboration gradient of the root economics space. *New Phytologist*, 234(3), 837–849. <https://doi.org/10.1111/nph.17906>
- Han, M., Chen, Y., Sun, L., Yu, M., Li, R., Li, S., Su, J., & Zhu, B. (2023). Linking rhizosphere soil microbial activity and plant resource acquisition strategy. *Journal of Ecology*, 111(4), 875–888. <https://doi.org/10.1111/1365-2745.14067>
- Hannula, S. E., Kielak, A. M., Steinauer, K., Huberty, M., Jongen, R., De Long, J. R., Heinen, R., & Bezemer, T. M. (2019). Time after time: Temporal variation in the effects of grass and forb species on soil bacterial and fungal communities. *MBio*, 10(6), 1–16. <https://doi.org/10.1128/mbio.02635-19>
- Hashimi, R., Kaneda, S., Kaneko, N., Ohta, H., & Komatsuzaki, M. (2020). Aggregate distribution and substrate-induced respiration under different tillage and mulching management systems in organic farming. *Soil Science and Plant Nutrition*, 66(6), 878–888. <https://doi.org/10.1080/00380768.2020.1824534>
- Hassink, J., Bouwman, L. A., Zwart, K. B., & Brussaard, L. (1993). Relationships between habitable pore space, soil biota and mineralization rates in grassland soils. *Soil Biology and Biochemistry*, 25(1), 47–55. [https://doi.org/10.1016/0038-0717\(93\)90240-C](https://doi.org/10.1016/0038-0717(93)90240-C)
- Henneron, L., Kardol, P., Wardle, D. A., Cros, C., & Fontaine, S. (2020). Rhizosphere control of soil nitrogen cycling: A key component of plant economic strategies. *New Phytologist*, 228(4), 1269–1282. <https://doi.org/10.1111/nph.16760>
- Kumar, A., Shahbaz, M., Blagodatskaya, E., Kuzyakov, Y., & Pausch, J. (2018). Maize phenology alters the distribution of enzyme activities in soil: Field estimates. *Applied Soil Ecology*, 125, 233–239. <https://doi.org/10.1016/j.apsoil.2018.02.001>
- Liberté, E. (2016). Below-ground frontiers in trait-based plant ecology. *New Phytologist*, 213(4), 1597–1603. <https://doi.org/10.1111/nph.14247>
- Laughlin, D. C. (2011). Nitrification is linked to dominant leaf traits rather than functional diversity. *Journal of Ecology*, 99(5), 1091–1099. <https://doi.org/10.1111/j.1365-2745.2011.01856.x>
- Legay, N., Clément, J. C., Grassein, F., Lavorel, S., Lemauviel-Lavenant, S., Personeni, E., Poly, F., Pommier, T., Robson, T. M., Mouhamadou, B., & Binet, M. N. (2020). Plant growth drives soil nitrogen cycling and N-related microbial activity through changing root traits. *Fungal Ecology*, 44(100910), 100910. <https://doi.org/10.1016/j.funeco.2019.100910>
- Lensi, R., Mazurier, S., Gourbière, F., & Jossierand, A. (1986). Rapid determination of the nitrification potential of an acid forest soil and assessment of its variability. *Soil Biology and Biochemistry*, 18(2), 239–240. [https://doi.org/10.1016/0038-0717\(86\)90035-0](https://doi.org/10.1016/0038-0717(86)90035-0)
- Li, Y., Clough, T. J., Moinet, G. Y. K., & Whitehead, D. (2021). Emissions of nitrous oxide, dinitrogen and carbon dioxide from three soils amended with carbon substrates under varying soil matric potentials. *European Journal of Soil Science*, 72(5), 2261–2275. <https://doi.org/10.1111/ejss.13124>
- Liebman, M., Baraibar, B., Buckley, Y., Childs, D., Christensen, S., Cousens, R., Eizenberg, H., Heijting, S., Loddo, D., Merotto, A., Jr., Renton, M., & Riemens, M. (2016). Ecologically sustainable weed management: How do we get from proof-of-concept to adoption? *Ecological Applications*, 26(5), 1352–1369. <https://doi.org/10.1002/15-0995>
- MacLaren, C., Bennett, J., & Dehnen-Schmutz, K. (2019). Management practices influence the competitive potential of weed communities and their value to biodiversity in south African vineyards. *Weed Research*, 59(2), 93–106. <https://doi.org/10.1111/wre.12347>
- Matson, P. A., & Vitousek, P. M. (1987). Cross-system comparisons of soil nitrogen transformations and nitrous oxide flux in tropical forest ecosystems. *Global Biogeochemical Cycles*, 1(2), 163–170.
- Meiss, H., Médiène, S., Waldhardt, R., Caneill, J., & Munier-Jolain, N. (2010). Contrasting weed species composition in perennial alfalfas and six annual crops: Implications for integrated weed management.

- Agronomy for Sustainable Development*, 30(3), 657–666. <https://doi.org/10.1051/agro/2009043>
- Mia, M. J., Monaci, E., Murri, G., Massetani, F., Facchi, J., & Neri, D. (2020). Soil nitrogen and weed biodiversity: An assessment under two orchard floor management practices in a nitrogen vulnerable zone in Italy. *Horticulturae*, 6(4), 96. <https://doi.org/10.3390/horticulturae6040096>
- Moreau, D., Bardgett, R. D., Finlay, R. D., Jones, D. L., & Philippot, L. (2019). A plant perspective on nitrogen cycling in the rhizosphere. *Functional Ecology*, 33(4), 540–552. <https://doi.org/10.1111/1365-2435.13303>
- Nguyen, D. B., Rose, M. T., Rose, T. J., Morris, S. G., & van Zwieten, L. (2016). Impact of glyphosate on soil microbial biomass and respiration: A meta-analysis. *Soil Biology and Biochemistry*, 92, 50–57. <https://doi.org/10.1016/j.soilbio.2015.09.014>
- Núñez, J., Orwin, K. H., Moinet, G. Y. K., Graham, S. L., Rogers, G. N. D., Turnbull, M. H., Clough, T. J., Dopheide, A., Davis, C., Grelet, G. A., & Whitehead, D. (2022). Mineralizable nitrogen and denitrification enzyme activity drive nitrate concentrations in well-drained stony subsoil under lucerne (*Medicago sativa* L.). *Applied Soil Ecology*, 176, 104499. <https://doi.org/10.1016/j.apsoil.2022.104499>
- Orwin, K. H., Buckland, S. M., Johnson, D., Turner, B. L., Smart, S., Oakley, S., & Bardgett, R. D. (2010). Linkages of plant traits to soil properties and the functioning of temperate grassland. *Journal of Ecology*, 98(5), 1074–1083. <https://doi.org/10.1111/j.1365-2745.2010.01679.x>
- Pérez-Harguindeguy, N., Díaz, S., Garnier, E., Lavorel, S., Poorter, H., Jaureguiberry, P., Bret-Harte, M. S., Cornwell, W. K., Craine, J. M., Gurvich, D. E., Urcelay, C., Veneklaas, E. J., Reich, P. B., Poorter, L., Wright, I. J., Ray, P., Enrico, L., Pausas, J. G., de Vos, A. C., ... Cornelissen, C. J. H. (2016). Corrigendum: New handbook for standardised measurement of plant functional traits worldwide. *Australian Journal of Botany*, 64(8), 715–716. [https://doi.org/10.1071/BT12225\\_CO](https://doi.org/10.1071/BT12225_CO)
- R Core Team. (2021). *R: A language and environment for statistical computing* (No. 4.1.1). R Foundation for Statistical Computing.
- Reich, P. B. (2014). The world-wide “fast-slow” plant economics spectrum: A traits manifesto. *Journal of Ecology*, 102(2), 275–301. <https://doi.org/10.1111/1365-2745.12211>
- Robson, T. M., Lavorel, S., Clement, J. C., & Roux, X. L. (2007). Neglect of mowing and manuring leads to slower nitrogen cycling in subalpine grasslands. *Soil Biology and Biochemistry*, 39(4), 930–941. <https://doi.org/10.1016/j.soilbio.2006.11.004>
- Roumet, C., Birouste, M., Picon-Cochard, C., Ghestem, M., Osman, N., Vrignon-Brenas, S., Cao, K. K., & Stokes, A. (2016). Root structure–function relationships in 74 species: Evidence of a root economics spectrum related to carbon economy. *New Phytologist*, 210(3), 815–826. <https://doi.org/10.1111/nph.13828>
- Santier, S., & Chamel, A. (1992). Penetration of glyphosate and diuron into and through isolated plant cuticles. *Weed Research*, 32(5), 337–347. <https://doi.org/10.1111/j.1365-3180.1992.tb01894.x>
- Šimek, M., & Cooper, J. E. (2002). The influence of soil pH on denitrification: Progress towards the understanding of this interaction over the last 50 years. *European Journal of Soil Science*, 53(3), 345–354. <https://doi.org/10.1046/j.1365-2389.2002.00461.x>
- Smith, M. S., & Tiedje, J. M. (1979). Phases of denitrification following oxygen depletion in soil. *Soil Biology and Biochemistry*, 11(3), 261–267. [https://doi.org/10.1016/0038-0717\(79\)90071-3](https://doi.org/10.1016/0038-0717(79)90071-3)
- Song, Y., Li, J., Zhong, L., Xue, J., Li, G., Qing, J., Rui, Y., Chen, G., Baying, T., & Li, F. Y. (2022). Short-term grazing rather than mowing stimulates N<sub>2</sub>O production potential through enhancing the bacterial pathway in semiarid grasslands. *Journal of Soils and Sediments*, 22(1), 32–42. <https://doi.org/10.1007/s11368-021-03051-5>
- Wang, J., & Zou, J. (2020). No-till increases soil denitrification via its positive effects on the activity and abundance of the denitrifying community. *Soil Biology and Biochemistry*, 142, 107706. <https://doi.org/10.1016/j.soilbio.2020.107706>
- Wright, I. J., Reich, P. B., Westoby, M., Ackerly, D. D., Baruch, Z., Bongers, F., Cavender-Bares, J., Chapin, T., Cornelissen, J. H. C., & Diemer, M. (2004). The worldwide leaf economics spectrum. *Nature*, 428(6985), 821–827. <https://doi.org/10.1038/nature02403>
- Zhang, C. J., Yang, Z. L., Shen, J. P., Sun, Y. F., Wang, J. T., Han, H. Y., Wan, S. Q., Zhang, L. M., & He, J. Z. (2018). Impacts of long-term nitrogen addition, watering and mowing on ammonia oxidizers, denitrifiers and plant communities in a temperate steppe. *Applied Soil Ecology*, 130, 241–250. <https://doi.org/10.1016/j.apsoil.2018.06.017>

## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**Figure S1.** Example of location for soil samples (10 samples pooled in one per vineyard), weed communities (4 samples), and soils to measure the potential for microbial activities (4 samples) in a vineyard.

**Figure S2.** (A) First two axes of a principal component analysis (PCA) based on management variables on the first two axes of the PCA. All the management variables are averaged over the 2015–2020 period. (B) Clustering of vineyards according to their positions in the first two dimensions of the PCA based on the management variables.

**Figure S3.** Soil characteristics of vineyards per management type.

**Figure S4.** Biomass, percentage of soil cover, richness and composition of weed communities.

**Figure S5.** (A) First and third axis of a PCA based on the aboveground community weighted means (CWM) and the root traits on the first and third dimensions of the functional PCA. (B) Weed community projection in the first and third axes of the functional PCA.

**Table S1.** Climate and soil characteristics of the vineyard network.

**Table S2.** Range of values of practice variables for the vineyards of the three main management types.

**Table S3.** Pairwise correlations between aboveground and belowground functional metrics at the community level.

**Table S4.** Standardised estimates of the soil abiotic characteristics (pH, SOM, %Sand, soil C:N), climate (aridity index) and management explaining weed functional parameters at the community level ( $n=15$ ).

**Table S5.** Standardised estimates of the weed functional properties (coordinates of the first two dimensions of the functional PCA) explaining the microbial functional properties ( $n=60$ ).

**Appendix S1.** Supplementary sections.

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