Pathways to persistence: plant root traits alter carbon accumulation in different soil carbon pools

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ABSTRACT

Aims: Mineral-associated organic matter, mainly derived from microbial by-products, persists longer in soil compared to particulate organic matter (POM). POM is highly recalcitrant and originates largely from decomposing root and shoot litter. Theory suggests that root traits and growth dynamics should affect carbon (C) accumulation into these different pools, but the specific traits driving this accumulation are not clearly identified.

Methods: Twelve herbaceous species were grown for 37 weeks in monocultures. Root elongation rate (RER) was measured throughout the experiment. At the end of the experiment, we determined morphological and chemical root traits, as well as substrate induced respiration (SIR) as a

proxy for microbial activity. Carbon was measured in four different soil fractions, following particle-size and density fractionation.

Results: In N_2-fixing Fabaceae species, root biomass, RER, root diameter, hemicellulose content and SIR, were all positively correlated with increased C in the coarse silt fraction. Root diameter and hemicellulose content were also negatively correlated with C in the POM fraction, that was greater under non N_2-fixing Poaceae species, characterized by lignin-rich roots with a high carbon:nitrogen ratio that grew slowly. The accumulation of C in different soil pools was mediated by microbial activity.

Conclusions: Our results show that root traits determine C input into different soil pools, mediated primarily by microbial activity, thus determining the fate of soil organic C. We also highlight that C in different soil pools, and not only total soil organic C, should be reported in future studies to better understand its origin, fate and dynamics.

Abbreviations

Abbreviation	*Meaning*
C	Carbon
POM	Particulate organic matter
C:N	Carbon – nitrogen ratio in plant tissue and/or soil
N_2-fixing	Dinitrogen fixing
t0	Time zero, beginning of the experiment
t37	Time 37 weeks, end of the experiment
ΔC	Delta carbon, as difference between carbon at time 0 and carbon at time 37, in different fractions (mg C g^{-1} soil)
C_{POM}	Carbon in the coarse POM 200-2000 µm fraction (mg C g^{-1} soil)
$C_{finePOM}$	Carbon in the fine POM 50-200 µm fraction (mg C g^{-1} soil)
C_{SILT}	Carbon in the 20-50 µm coarse silt fraction (mg C g^{-1} soil)
$C_{SILT+CLAY}$	Carbon in the fine silt+clay <20 µm fraction (mg C g^{-1} soil)
ΔC_{SUM}	Sum of delta carbon in different fractions, $\Delta C_{SUM} = \Delta C_{POM} + \Delta C_{finePOM} + \Delta C_{SILT} + \Delta C_{SILT+CLAY}$ (mg C g^{-1} soil)
RER	Root elongation rate (mm d^{-1})
RLP	Root length production (m)
RER_{NEW}, RLP_{NEW}	RER and RLP of 'new' roots initiated during the 2 weeks interval between measurements
RER_{OLD}, RLP_{OLD}	RER and RLP of 'old' roots, initiated more than 2 weeks before the measurement

SIR	Substrate induced respiration (μg $C\text{-}CO_2$ g^{-1} soil h^{-1})
PCA	Principal component analysis

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INTRODUCTION

Given the current climate change emergency, several international initiatives have been launched to unlock the potential of soils to sequester atmospheric carbon (C) (e.g. 4 per Thousand Initiative, Minasny et al. 2017). Better understanding the interactions between vegetation and soil has become central for sequestering C into anthropogenically disturbed soil, such as agricultural fields, mining waste soil, road embankments and technosols (Paustian et al. 2016; Griscom et al. 2017). Plants act as a major conduit for transferring C into soils via litterfall, root mortality and exudation (Six et al. 2004; Derrien et al. 2016; Sokol et al. 2019). Some C is transformed by soil microbes and released back into the atmosphere by respiration (Jones et al. 2009; Kuzyakov and Larionova, 2005), but C can also be stabilized in soil, increasing its residence time (Besnard et al. 1996; Lal, 2004; Rasse et al. 2005; Bardgett et al. 2014 ; Vidal et al. 2018; Sokol et al. 2019). Carbon persists in soil at different time scales based on recalcitrance (short-term preservation), spatial inaccessibility to decomposers due to occlusion in soil aggregates, and adsorption to mineral and metal surfaces (Kleber et al. 2011, Schmidt et al. 2011, Poirier et al. 2018). These mechanisms are influenced by abiotic and biotic factors and especially by plant roots, since their C is preferentially stabilized compared to aerial parts (Balesdent and Balabane, 1996; Rasse et al. 2005, Kätterer et al. 2011). In a recent review, Poirier et al. (2018) argued that the root traits most influencing C stabilization are those related to chemical composition, root exudation and the presence of symbionts (e.g. mycorrhizas and *Rhizobium* bacteria), whereas the role of morphological traits is not yet clear. More specifically, root traits increasing chemical recalcitrance promote short-term C stabilization by slowing root decomposition rates, whereas root traits increasing exudation rate promote long-term C stabilization faster. Several studies have analysed the link between plant functional traits, microbial activity and C accumulation (Chapin 2003; Lavorel et al. 2007; De Deyn et al. 2008; Poirier et al. 2018). However, as yet, it is not understood how root traits can alter the accumulation and potential persistence of C.

Through differences in chemical and physiological traits, roots should affect C accumulation into different C pools depending on soil texture. These pools are defined as: i) coarse particulate organic matter (coarse POM, > 200µm fraction), that is free in the soil at different levels of degradation, ii) fine POM (50-200µm fraction), that comprises free organic C and organic C occluded in soil macroaggregates. These two pools are mostly derived from the decomposition of roots and shoots (Kögel-Knabner, 2002), and their short-term C protection from microbial consumption relies mainly on the recalcitrance of their lignocellulose C structures and the physical protection given by macroaggregate structure (Six et al. 2002). Finally, iii) C protected in the coarse silt and fine silt+clay pools (20-50µm and <20µm fractions, respectively); that is highly processed and protected from microbial consumption via occlusion in microaggregates and through organo-mineral adsorption to

clay particles and metals. This C is mostly derived from dissolved organic C originating from the degradation of above and belowground plant C input (Bird et al. 2008; Rubino et al. 2010; Sanderman et al. 2014), from root exudation of labile rhizospheric compounds and from microbial compounds (Simpson et al., 2007; Mambelli et al., 2011; Cotrufo et al. 2013; 2014; Vidal et al. 2018; Rossi 2019). It is now generally accepted that labile low molecular weight compounds persist in soil longer than chemically recalcitrant C structures, when protected by organo-mineral adsorption (Mikutta et al. 2006; Kleber et al. 2015; King et al. 2019; Robertson et al. 2019; Sokol et al. 2019). The stability of sequestered C in soil is therefore linked to the fraction of soil to which it is associated, with a greater stability of C pools associated with finer fractions (Torn et al., 2009).

As C accumulation into the coarse POM pool is related to the amount of recalcitrant matter present, it should therefore be greater in soils containing roots with high cellulose, lignin and carbon:nitrogen ratio (C:N) (Poirier et al. 2018). However, it is C-rich exudates produced during fine root elongation that promote long-term C stabilization in the coarse silt and fine silt+clay fractions (Mikutta et al. 2006; King et al. 2019; Robertson et al. 2019; Sokol et al. 2019), and together with mucilage and border cells (shed during growth), are important substrates for microbial communities (Dennis et al., 2010). These C substrates that are assimilated by microorganisms close to the root apex are utilized rapidly for respiration and growth, or lost as microbial exudates or exopolysaccharides that are then used as a substrate for subsequent microbial communities. Since microbial byproducts (from activity in any soil C pool) are believed to be the main precursor of protected C due to organo-mineral interactions (Simpson et al., 2007; Mambelli et al., 2011; Cotrufo et al., 2013; Rossi 2019), root exudation should influence the C storage in this fraction. It is however important to mention that exudation and the resulting microbial activiy can also negatively influence soil C storage, increasing the consumption of preexistent soil C (i.e. priming effect; Hamer and Marschner, 2005; Shahzad et al. 2017). Root traits related to exudation are however poorly understood; the few studies available showed contrasted results and relate to root morphological traits measured at the whole root system level (Roumet et al. 2006; Guyonnet et al. 2018). Because root exudates are mainly released at the elongating root tip, where rhizospheric microbial activity is high (Jones et al. 2009; Canarini et al. 2019), it can be expected that root elongation rate (RER) is a powerful predictor of C deposits in coarse silt and fine silt+clay C pools (Holz et al. 2018). Root elongation rate is affected principally by local abiotic soil conditions such as soil temperature, moisture, and compaction, but also differs among species, although most known data is related to woody species (Steinaker et al. 2011; Mohamed et al. 2016; Wang et al. 2018). Fast growing species with small diameter fine roots, high specific root length and N uptake rate usually have high RER (Larson and Funk 2016).

Determining plant traits that increase C accumulation in different soil C pools should therefore enable the selection of species that promote C persistence in soil. Recent debate has focused on the ability of dinitrogen fixing (N_2-fixing) species to sequester large amounts of C in soil (Plaza-Bonilla et al. 2016; King et al. 2018). Bacteria such as *Rhizobium*, present in nodules of N_2-fixing species, produce large amounts of exopolysaccharides (Downie, 2010; Sasse et al. 2018), that are adsorbed onto fine silt and clay particles (Fehrmann and Weaver, 1978). Also, N_2-fixing species have roots that are easily degradable with a high content of hemicelluloses (Hernández et al. 2017) and low C:N ratio (Warembourg et al. 2003; Roumet et al. 2005), therefore enhancing microbial activity (Poirer et al. 2018). However, it is not known whether N_2-fixing species promote greater C accumulation in the fine silt and clay soil fractions, thereby enhancing C persistence in soil.

We explored the effect of root traits on C accumulation into different soil C pools beneath 12 herbaceous species grown in monocultures for 37 weeks. These species had diverse root traits in terms of morphology, chemical composition, and elongation rate and belonged to different plant families: five N_2-fixing Fabaceae, five Poaceae, one Rosaceae and one Plantaginaceae. Our main hypothesis is that C accumulation into different soil C pools is driven by root traits and their effects on microbial activity and biomass. More specifically, we hypothesize that (i) traits related to high RER promote C accumulation in the coarse silt and fine silt + clay C pools, since these traits are expected to favour exudation and subsequent microbial activity, whereas (ii) root traits related to chemical recalcitrance (high lignin and cellulose content and high C:N ratio), promote C accumulation in the unprotected POM pool, and iii) N_2-fixing species favour C accumulation in the coarse silt and fine silt + clay pools. Results should enable us to disentangle the relationships between root growth, traits and the accumulation and stabilization of C in different soil C pools, between different families (Poaceae and Fabaceae) and N_2 and non N_2-fixing species.

MATERIALS AND METHODS

Experimental setup

The experiment was set up in the experimental garden of CEFE-CNRS Montpellier, France (43.6389° N°, 3.864125° E and lasted 37 weeks (from t0: Sept-2016 to t36: July-2017). Twelve herbaceous species were grown as monocultures in steel boxes (0.7 m length x 0.7 m width x 0.3 m depth): five N_2-fixing species from the Fabaceae family and seven non N_2-fixing species, including five Poaceae, one Plantaginaceae and one Rosaceae species (Table 1). A weather station was set up permanently in the

146 experimental garden, and air humidity, air temperature (minimum, maximum and mean daily) and
147 solar irradiation (measured daily) were monitored throughout the experiment.

148

149 Seventy-eight boxes were prepared: six replicate boxes per species and six additional boxes of bare
150 soil used as controls. Boxes were organized in three blocks with two rows of 13 adjacent boxes in each
151 block, and with a distance of 50 cm between each box. Each row comprised 12 monocultures (one per
152 species) and a bare soil, randomly arranged in each row (Fig S1). Boxes of the first row were used for
153 destructive plant and soil sampling, while the boxes of the second row were equipped with rhizotrons
154 for the study of root elongation (Section 1.1, Fig. S1). In the second block, each box was equipped with
155 soil temperature and humidity sensors placed at a depth of 0.1 m. Soil temperature was recorded
156 every 4 hours with an i-button sensor (iButtonLink, Wisconsin, USA); soil relative humidity was
157 recorded every hour with moisture sensors (Waterscout SM100, Spectrum Technologies Inc.) and a
158 datalogger (WatchDog weather station 200 series, Spectrum Technologies Inc.). These boxes were
159 undisturbed for the duration of the experiment. Rhizotrons comprised a 0.2 m width x 0.3 m depth x
160 0.05 m thick pane of transparent plexiglass set into the lower walls of the boxes, through which roots
161 were observed and root elongation rate (RER, in mm $root^{-1}$ day^{-1}) and root length production (RLP, in
162 mm mm^{-2} day^{-1}), were calculated (Fig. S2). For RER and RLP, only one replicate box per species was
163 analyzed, because the analysis of root images was extremely time consuming.

164 Boxes were inclined at 20° relative to the horizon to encourage the positive geotropism of roots when
165 they came into contact with rhizotron windows (Huck and Taylor, 1982). Boxes were filled with soil
166 sieved to 8 mm. Five layers of soil were successively added and manually compacted to attain a volume
167 of 0.113 m^3, i.e., a total of 190 kg of soil per box (bulk density = 1.70 ± 0.02 g cm^{-3}). The soil, excavated
168 in Villefort (France; 44°26'25" N, 3°55'58" E), was sandy-loam (62.6 % sand, 26.1 % silt, 11.3 % clay);
169 with 1.36 g kg^{-1} of total N, 16.9 g kg^{-1} of total C, 0.069 g kg^{-1} of phosphorus (P Olsen), pH in water was
170 7.06, and cation-exchange capacity (CEC) was 7.98 $cmol_c$ kg^{-1}.

171 On 17-19 October 2016, 72 boxes were sown as monocultures (12 species x 6 replicates with one
172 replicate species per row). Seeds of each species were sown in lines, the distance between lines was
173 75 mm and the distance between plants within a line was 75 mm, leading to a final plant density of
174 155 plants m^{-2}. Once seeds had germinated, each box was inoculated with a purified solution of local
175 *Rhizobium* bacteria strains (Incolum Valorhiz™, France) and was netted to avoid birds disturbing seeds.
176 During the experiment, mean air temperature was 13 C° (Figure S3) with a maximum of 30 °C and a
177 minimum of -0.4 °C (Figure S3a), and the cumulative precipitation was 349 mm (Fig S3 for additional
178 information on climatic conditions over the 37 weeks of the experiment). Soil temperature in the boxes

179 followed closely the air temperature over the 37 weeks period, with a mean of 13.5 °C, a maximum of
180 25.9 °C and a minimum of 3 °C (Figure S3a). Air humidity ranged from 53 to 87%, with a mean value of
181 74% (Figure S3a) and solar irradiation ranged from 320 to 897 W m^{-2} with a mean value of 568 W m^{-2}
182 (Figure S3b). During the experiment, boxes were carefully weeded by hand and plants were cut to
183 ground level every 4 months to maintain a regular aerial cover. In addition, each box was watered with
184 sprinklers when required.

185

186 *Analysis of carbon content in different soil fractions*

187 Soil C content was measured before filling the boxes, as a reference for time 0 (t0), on three samples
188 from the initial homogenized soil batch, and at the end of the experiment, i.e. at 37 weeks (t37) after
189 sowing. At t37, soil samples were taken at 0-200 mm depth using a soil corer (75 mm in diameter) in
190 each box dedicated to soil and plant sampling. All soil samples were separated into two depths (0-100
191 mm and 100-200 mm), air dried and separately sieved to 2 mm. A subsample of 40 g of soil was
192 collected at a depth of 0-100 mm for subsequent fractioning into POM fractions (coarse POM: 200-
193 2000 μm and fine POM: 50-200 μm), coarse silt (20-50 μm), fine silt + clay (<20 μm) fractions. Soil
194 fractionation was carried out using the method from Gavinelli et al. (1995). Soil samples were
195 presoaked overnight in 300 ml of deionized water at 4°C with 0.5 g of hexametaphosphate to enhance
196 disaggregation. Soil was then shaken at 300 rpm (digital orbital shaker, Intertek) with five agate
197 marbles for 2 h (i.e., the time suggested for sandy soils, to avoid the transfer of C into finer fractions,
198 Gavinelli et al. 1995). The soil was wet sieved with a 200 μm sieve, and the resulting 200-2000 μm
199 fraction was then transferred into a separate container and soaked in deionized water. The floating
200 coarse particulate organic matter (POM) was then carefully collected. The remaining 200-2000 μm
201 fraction represented the coarse sand fraction in soil and was carefully collected by washing the content
202 of the sieve in a beaker using deionized water. Then, the remaining fraction was sieved with a 50 μm
203 sieve, to separate and collect the fine sand fraction and the fine POM fraction (50-200 μm). The
204 remaining fraction <50 μm was sonicated with a 1510E-MT Bransonic sonicator for 10 minutes to break
205 microaggregates before sieving at 20 μm. The 20-50 μm fraction (coarse silt) was collected and the
206 resulting solution of deionized water and <20 μm fraction collected in a beaker and filled up to 1.0 L.
207 This solution was tumbled 30 times to homogenize it and an aliquot of 100 mL was collected with the
208 aid of a syringe, representing the fine silt + clay fraction. All the fractions were oven dried at 40°C until
209 all the water evaporated. The dried fractions were weighed to check that the sum of the fraction's
210 weight did not differ from +/-5% the initial 40 g total weight. The quality of the soil particle dispersion

was checked and did not differ +/-5% compared to the soil texture analysis, being 62.6% in the sand fraction and 37.4% in the fine silt + clay fraction.

Carbon content in each of the soil fractions (C_{POM} - carbon in the coarse POM fraction; $C_{finePOM}$ – carbon in the fine POM fraction; C_{SILT} - carbon in the coarse silt fraction and $C_{SILT+CLAY}$ - carbon in the fine silt+clay fraction), was analyzed using an elemental analyzer (CHN model EA 1108; Carlo Erba Instruments, Milan, Italy) to assess the amount of C present in each pool. A subsample of 0.1 g was taken from each 40 g sample and analyzed without fractioning to determine the total C in the bulk sample. The difference between total C in bulk soil and the sum of C in the different soil fractions was used to assess the correctness of the fractionation (+/-5%) and was equal to 97.3% (SD=22%; n=34).

We calculated soil C changes (ΔC) in each soil fraction as the difference between C content in mg C g^{-1} soil at 0 and 37 weeks ($\Delta C = C_{t37} - C_{t0}$). The sum of ΔC in each soil fraction ($\Delta C_{SUM} = \Delta C_{POM} + \Delta C_{finePOM} + \Delta C_{SILT} + \Delta C_{SILT+CLAY}$) was also calculated to investigate the variation in the totality of the soil fractions. Note that ΔC can be either positive (accumulation) or negative (depletion due to the positive priming effect, that is the increase of pre-existing soil C consumption and losses due to vegetation, Kuzyakov, 2002).

Measurement of root elongation rate (RER) and root length production (RLP)

As soon as the first root of a given species was visible in a rhizotron, roots of that species were scanned every 2 weeks for the experiment experiment (i.e. n = 19 measurement dates) using a smartphone scanner application CamScanner (INTSIG Information Co., Ltd, Shanghai, China; version 3.9.5). A smartphone (Samsung Xcover3, Samsung Electronics, Korea) was kept at a fixed distance from the rhizotron (0.3 m) and a ruler was included in the picture to set the scale (Mohamed et al. 2017). Images were then analyzed with the SmartRoot software (Lobet et al. 2011), a freeware plugin of ImageJ software (Schneider et al. 2012). The images acquired were converted into 8-bit grey scale and, when necessary, color- inverted, so that roots were dark on a lighter background. SmartRoot allows the semi-automatic tracing of roots by clicking on the basal point of each root (Fig. S4a). Data extracted include the length and diameter of the roots. The resulting traced image of roots could then be imported and superimposed onto a new image, allowing analysis of subsequent images and creating a time-dependent dataset acquiring root length at different time steps.

Root elongation rate (RER; mm d^{-1}) is indicative of primary root growth and is defined as the difference in individual root length measured between two dates. RER is a frequent but punctual observation of root dynamics over time. As soil C storage is a cumulative process, root length production (RLP; m)

after 37 weeks was also calculated for up to 60 roots (randomly chosen) per rhizotron. RLP is the total length of all roots produced in a specific period of time (Mommer et al. 2015). Of these 60 roots measured, 30 were selected from the 'new roots', i.e., the roots that were not present in the previous scan, and so had formed in the previous two weeks. Of the 30 'new roots', 20 were primary and first order roots and ten were second order laterals (Fig. S4a, according to the developmental centrifugal protocol of root topology; Berntson, 1997). Then, 30 'old roots' were selected at each subsequent sampling date. The 'old roots' were the roots already present in the previous scan (again, 20 primary axes and ten second order lateral roots). Fig. S5 shows an example of rhizotron analysis for new and old roots. To have a more representative sample of the 'old roots,' ten primary roots were selected from the 20 primary 'old roots' of the previous scan, ten were selected from the 20 newly emerged roots of the previous scan, five were chosen from the ten second order lateral 'old roots' of the previous scan and five were selected from the newly emerged second order laterals of the previous scan. This method was used to select roots at each subsequent sampling date. If one or more roots had: 1) reached the boundaries of the rhizotron, or 2) were in a bundle and not distinguishable (Fig. S4b), or 3) could not be analyzed for any other reason (e.g. soil masking the root), they were discarded and different roots were then selected.

The mean daily RER was calculated by subtracting from the length of a root (L_{t2}) the length of the root acquired at the previous sampling date (L_{t1}). This result was then standardized dividing by the number of days between the two sampling dates (t) to have the mean elongation rate of a single root:

$$RER(t) = (L_{t2} - L_{t1})/[t2 - t1] \quad \text{Equation 1}$$

Root length production (RLP) of roots over the 37 weeks was chosen as a cumulative indicator for root dynamics, adapted from Mommer et al. (2015):

$$RLP = \sum_{t=1}^{T} \sum_{r=1}^{R} (\mathrm{RER(t)} \quad * \frac{R_{30}}{R}) \quad \text{Equation 2}$$

Where *t* represents the sampling date ; $_{(t)}$; RER(t) is the daily RER; R the real number of roots analyzed in that interval. Since the number of analyzed roots varied depending on dates and species, we decided to standardize the analysis of RLP for R_{30} = 30 roots.

To refine the understanding of root dynamics, the RER and RLP were calculated separately for the new roots (RER_{NEW} and $RLP_{NEW,}$ i.e. roots initiated during the 2 weeks interval between measurements), old roots (RER_{OLD} and RLP_{OLD}, i.e. roots older than 2 weeks), and also the total root system, regardless of root age. For all species, RER was high during the first two samplings after their initiation and then decreased rapidly or stopped. Therefore, mean RER could be biased by the development of new roots, justifying our decision to separate roots based on age and order for the statistical analysis.

Analysis of root traits

After 37 weeks, a soil core (75 mm diameter, 200 mm depth), centered on one individual plant per species and per box was collected. In each core, roots were separated from the aboveground part and washed. Roots were sorted into absorptive roots, typically the first, second and third root orders (defined as the most distal root orders), and transport roots, that were higher order roots (all orders above third order roots, following McCormack et al. 2015). A subsample of absorptive roots (0.1 g dry mass on average) was selected, stained in a solution of methyl violet (0.5 g L^{-1}), spread into a transparent water filled tray and scanned at 800 dpi (Epson Expression 1680, Canada).

The software Winrhizo Pro (Regent Instruments, Quebec, Canada) was used to determine the root diameter (from 0 to 2 mm, with a 0.1 mm diameter interval) of absorptive roots. Roots were then oven dried at 40°C for 3 days and weighed to determine the total root dry mass for each core.

For each species, determination of root chemical composition was conducted on three subsamples of absorptive roots reserved for chemical analyses. C and N concentrations were determined on ground material using an elemental analyser (CHN model EA 1108; Carlo Erba Instruments, Milan, Italy). Concentrations of water-soluble compounds + hemicelluloses, cellulose and lignin were obtained following the Van Soest method (Van Soest, 1963) and using a fiber analyser (Fibersac 24; Ankom, Macedon, NJ, USA). This method consists of measuring the various plant tissue constituents by sequential extraction with neutral detergent, acid detergent and sulfuric acid (76%).

Substrate-induced respiration (SIR) was used as a proxy for potential soil microbial respiration and activity, according to Beare et al. (1990). Briefly, 20 g air-dried 2 mm sieved soil samples were incubated in 150 mL sealed serum flasks with 1.5 mg C-glucose g^{-1} soil, at 80 % field capacity and at 25°C. A 200 µL aliquot of the flask headspace was analyzed for CO_2 concentration after 2 and 6 hours using a microcatharometer (MicroGC Serie S, SRA Industries, Marcy l'Etoile, France), equipped with a PoraPlot column (Agilent, Santa Clara, United States). Substrate induced respiration rates were calculated as the mass of C-glucose converted to C-CO_2 per g of soil dry weight and per hour (in µg C-CO_2 g^{-1} soil h^{-1}).

Statistical analysis

First, a one-way analysis of variance (ANOVA) and post-hoc Tukey honestly significance difference (HSD) tests were performed to test the effects of species on mean RER, mean RLP, root traits and ΔC sequestration in soil C pools. Secondly, one way ANOVAs were conducted on the five Poaceae species and the five Fabaceae species, i.e excluding *P. lanceolata* and *S. minor* (hypothesis 3). In order to select

the environmental parameters to be included in the constrained ordination, an initial db-RDA including all parameters was performed followed by a stepwise model selection using Generalized Akaike Information Criterion (AIC, ordistep function with a backward direction). The normal distribution of residuals was verified using a Shapiro-Wilk test ($p = 0.05$). If the data were not normally distributed, one way ANOVA was substituted with a Kruskal-Walls test. Finally, the same procedure using one way ANOVA was performed to compare the mean effect of N_2-fixing and non N_2-fixing species (for the latter, grouping together Poaceae, *P. lanceolata* and *S. minor*) on root traits and C storage (hypothesis 3).

A principal component analysis (PCA) was performed on 12 variables (six root traits, four ΔC of each soil C pool plus their sum, and SIR) using the mean for three replicate boxes (n = 12) to investigate the effect of root traits at the species level. RER and RLP were not included in the PCA since they were measured on one replicate box per species. Then, Pearson's correlation coefficients were calculated to study the relationships between root traits and ΔC in each soil C pool (hypotheses 1 and 2) and linear models of the significant correlations were analyzed to study the data dispersion. To deepen the undesrtanding of these correlations, Pearson's correlation analysis and a study of the linear models were performed on raw data (n = 34) to study relationships at the individual level.

To investigate the effect of abiotic factors on root growth dynamics, Pearson's correlation coefficients were calculated between mean daily RER, mean RLP, mean daily soil and air temperatures, mean daily soil humidity and mean daily solar irradiation (n = 12 for each variable). Means of daily climate data were calculated for the 2 weeks preceding the measurement of RER.

All the statistical analyses were performed in the open-source statistical environment R, version 3.4.3 (R Development Core Team, 2017) using the packages *Hmisc* (Harrel 2007) and *vegan* (Oksanen et al. 2019).

RESULTS

Effect of plant species on soil carbon accumulation (ΔC) in different C pools associated with soil fractions

Plant species did not significantly influence the accumulation of C in different pools, nor in the sum of C pools (Fig. 1). The mean ΔC_{SUM} increase was 1.72 ± 1.45 mg C g^{-1} soil, and was highest in soil beneath *L. corniculatus* (3.60 ± 0.70 mg C g^{-1} soil) compared to the bare soil control (0.21 ± 3.87 mg C g^{-1} soil, Fig. 1a). The mean increase in the coarse pool ΔC_{POM} was 0.58 ± 0.34 mg C g^{-1} soil (Fig. 1b) and in the $\Delta C_{finePOM}$ was 1.21 ± 0.74 mg C g^{-1} soil (Fig. 1c). In the protected C_{SILT} pool, the ΔC mean increase was 0.57 ± 0.34 mg C g^{-1} soil (Fig. 1d), while the $\Delta C_{SILT+CLAY}$ decreased by -0.50 ± 0.77 mg C g^{-1} soil (Fig. 1e).

However, no significant differences were found between any species and bare soil with regard to any C pool (Fig. S6, C data in different soil C pools for each species at t37).

Significant differences in mean ΔC between N_2-fixing Fabaceae and non N_2-fixing Poaceae were found with regard to C_{POM} and C_{SILT}. Mean C_{POM} was significantly higher in soil beneath Poaceae species (ANOVA, p = 0.024, Tukey HSD test, Fig. 2a), whilst C_{SILT} was significantly higher in Fabaceae species (ANOVA, p = 0.060, Tukey HSD test, Fig. 2b), and no significant differences were found in C_{SILT} between Poaceae and bare soil. When grouping the data for all the non N_2-fixing species (i.e., Poaceae, *P. lanceolata* and *S. minor*), mean C_{POM} was higher compared to N_2-fixing Fabaceae (ANOVA, p = 0.06, F = 3.61) but C_{silt} was lower (ANOVA, p =0.01, F = 7.01) (Fig. 2), although a Tukey HSD test did not find significant differences between N_2-fixing and non N_2-fixing species.

Root elongation rate (RER) and root length production (RLP)

More than a threefold variation in mean daily RER_{TOT} occurred among species, ranging from 0.23 mm d^{-1} (*F. rubra*) to 0.75 mm d^{-1} (*T. repens*) (Table 1). Mean daily RER_{TOT} did not differ between N_2-fixing Fabaceae (0.57 ± 0.08 mm d^{-1}on average) and non N_2-fixing Poaceae (0.42 ± 0.13 mm d^{-1}, ANOVA, p = 0.221, Table 1), even when grouped with non N_2-fixing species (0.46±0.14 mm d^{-1}, ANOVA, p = 0.075). Mean daily RER_{TOT} peaked at 0.75 mm d^{-1} in mid-February for Poaceae and then decreased, attaining a value of 0.4 mm d^{-1} from April to June 2017 (Figs. S7). For Fabaceae species, mean daily RER_{TOT} peaked at 1.1 mm d^{-1} in May 2017, before decreasing sharply in June 2017 (Fig. S7, mean RER_{TOT} for Fabaceae and Poaceae species).

The mean daily RER for new roots (RER_{NEW}, 0.83 ± 0.22 mm d^{-1}) was significantly higher than that of old roots (RER_{OLD}, 0.17 ± 0.09 mm d^{-1}, ANOVA, p < 0.001). Mean daily RER_{NEW} ranged from 0.32 mm d^{-1} (*F. rubra*) to 1.13 mm d^{-1} (*D. glomerata*) whereas RER_{OLD} ranged from 0.05 mm d^{-1} (*P. pratensis*) to 0.40 mm d^{-1} (*T. pratense*). Mean daily RER_{NEW} did not differ in N_2-fixing Fabaceae compared to non N_2-fixing Poaceae or all non N_2-fixing species grouped together. However, mean daily RER_{OLD} was greater in N_2-fixing Fabaceae (0.25 ± 0.09 mm d^{-1}) than in non N_2-fixing Poaceae (0.13 ± 0.03 mm d^{-1}, ANOVA, p = 0.020) or all non N_2-fixing species grouped together (0.12 ± 0.04 mm d^{-1}, ANOVA, p = 0.005, Table 1).

After 37 weeks, the highest cumulative RLP_{TOT} was observed in in *O. viciifolia* (3.62 m) and the lowest in *F. rubra* (1.19 m) (Table 1). N_2-fixing Fabaceae species possessed a greater RLP_{TOT} (3.37 ± 2.32 m) compared to non N_2-fixing Poaceae (2.32 ± 0.70 m, ANOVA, p = 0.032), as well as all the N_2-fixing species grouped together (2.42 ± 0.63 m, ANOVA, p = 0.009). Root dynamics of only three species were correlated with climate factors. In *L. corniculatus,* mean daily RER_{TOT}, RER_{OLD}, RER_{NEW}, RLP_{TOT}, RLP_{OLD} and

371 RLP_{NEW} were all positively correlated with soil and air temperature and solar irradiation (Tables S1, S2).
372 In *T. repens,* RER_{TOT} and RER_{NEW}, RLP_{NEW} were significantly and positively correlated with soil and air
373 temperature (Tables S1, S2). With regard to Poaceae species, mean RER_{NEW} of *D. glomerata* was
374 negatively correlated with soil and air temperature and solar irradiation (Table S1). In *O. viciifolia*,
375 RLP_{TOT} was slightly and positively correlated with solar irradiation (Table S2). In *D. glomerata*, RLP_{NEW}
376 only, was negatively correlated with soil and air temperature (Table S2).

377

378 *Root biomass, diameter and chemical composition*

379 At 37 weeks, *M. sativa* had significantly greater mean root biomass (4.23 ± 0.42 g) compared to all
380 other species (Tukey HSD test, Table 1). In general, N_2-fixing Fabaceae species had a significantly higher
381 mean root biomass (2.08 ± 1.33 g) compared to non N_2-fixing Poaceae (0.62 ± 0.11 g) and all the non
382 N_2-fixing species grouped together (0.65 ± 0.17 g, ANOVA, $p < 0.001$). The mean diameter of absorptive
383 roots differed significantly between species, with *O. viciifolia* having the thickest absorptive roots and
384 *D. glomerata* the thinnest (0.21 ± 0.14 mm, Table 1). Species from the N_2-fixing Fabaceae family had
385 significantly thicker absorptive roots (0.39 ± 0.11 mm) compared to non N_2-fixing Poaceae (0.23 ± 0.03
386 mm) or all the non N_2-fixing species grouped together (0.25 ± 0.03 mm, ANOVA, $p < 0.001$).

387 The chemical composition of absorptive roots strongly varied among species and between N_2-fixing
388 Fabaceae and non N_2-fixing Poaceae or all the non N_2-fixing species grouped together (Table 1).
389 Absorptive roots of N_2-fixing Fabaceae possessed more hemicelluloses + water-soluble compounds
390 (705 ± 74 mg g^{-1}) than non N_2-fixing Poaceae (543 ± 33 mg g^{-1}) or all non N_2-fixing species grouped
391 together (583 ± 69 mg g^{-1}), a lower mean lignin content (N_2-fixing Fabaceae: 173 ± 56 mg g^{-1}, non N_2-
392 fixing Poaceae: 302 ± 59 mg g^{-1}, all non N_2-fixing species grouped together: 264.18 ± 79.06 mg g^{-1}), and
393 a lower mean C:N ratio (N_2-fixing Fabaceae: 19.15 ± 3.07, non N_2-fixing Poaceae: 58.67 ± 6.34 , and all
394 non N_2-fixing species grouped together: 62.04 ± 7.41). Mean root cellulose content did not differ either
395 among species or between N_2-fixing Fabaceae and non N_2-fixing Poaceae. However, when all the non
396 N_2-fixing species were grouped together, absorptive roots had a significantly higher mean cellulose
397 content compared to N_2-fixing Fabaceae (Table 1).

398

399 *Soil substrate induced respiration (SIR)*

400 Mean SIR for soil microbial communities varied significantly among species and between N_2-fixing
401 Fabaceae (5.28 ±1 µg $C\text{-}CO_2$ g^{-1} soil h^{-1}) and non N_2-fixing Poaceae (3.12 ± 0.41 µg $C\text{-}CO_2$ g^{-1} soil h^{-1},
402 ANOVA, $p < 0.001$, Table 1). Mean SIR ranged from 2.47 ± 0.34 µg $C\text{-}CO_2$ g^{-1} soil h^{-1} (beneath *B. erectus)*

to 6.41 ± 0.56 µg $C\text{-}CO_2$ g^{-1} soil h^{-1} (beneath *M. sativa*). When grouping all the non N_2-fixing species together, mean SIR was still significantly lower (3.1 ± 0.46 µg $C\text{-}CO_2$ g^{-1} soil h^{-1}) compared to N_2-fixing Fabaceae (5.28 ± 1.02 µg $C\text{-}CO_2$ g^{-1} soil h^{-1}, ANOVA, $p < 0.001$, Table 1).

Relationships between soil carbon accumulation (ΔC), root growth dynamics, root traits, and substrate induced respiration (SIR)

The PCA conducted on the ΔC in the different C pools, SIR and root traits explained 64.6% of the variance of the variables analyzed (Fig. 3). The first PCA axis (horizontal), accounting for 44.4% of the variation, opposed ΔC_{POM} (negative) and ΔC_{SILT} (positive), while the remaining C pools, as well as the sum of C pools, covaried and were quite orthogonal to ΔC_{POM} and ΔC_{SILT} and related to the second PCA axis, that accounted for 20.2% of the variation. SIR and root biomass, diameter, and hemicelluloses + water soluble compounds content of absorptive roots all went along the 1st axis (positive) together with ΔC_{SILT}. Root traits linked with recalcitrance, lignin, cellulose and C:N ratio, went along the 1st axis (negative) together with ΔC_{POM}. Convex hull polygons reflecting intraspecific variations generally had small areas and were segregated over the biplot (Fig. 3). The PCA strongly discriminated Poaceae from Fabaceae. Poaceae were all on the negative end of the first axis and were characterized by high lignin and cellulose contents, high C:N and accumulation of C in the coarse POM fraction. Fabaceae species were at far right of the first axis and were characterized by a higher biomass and thicker roots that were rich in hemicelluloses, favoring accumulation of C in the coarse silt fraction. The two other non N_2-fixing species were situated in intermediate positions on the axis.

When analyzing the species effect of root traits on C storage (n = 12) regression analyses showed that mean ΔC_{POM} was not related to RER, but was slightly significantly and negatively related to two traits: diameter and hemicelluloses + water-soluble compounds content of absorptive roots (Table S3a, Figure 4a,b). Mean ΔC_{SILT} was significantly and positively correlated with mean daily RER_{OLD} and RLP_{OLD} and with the mean diameter of absorptive roots, root biomass, hemicellulose + water-soluble compounds of absorptive roots, and SIR (Table S3s, Figures 5a,b,c,d,f, h), whereas mean ΔC_{SILT} was negatively correlated with mean lignin and C:N ratio (Table S3s, Figures 5e,g). Linear regressions of mean ΔC_{SILT} and C:N ratio show two segregated clusters of points: one with low C:N related to N_2-fixing species and one with non N_2-fixing species having a high C:N ratio and low accumulation in ΔC_{SILT} (Figure 5g). Variations in mean ΔC_{SUM}, $\Delta C_{finePOM}$ and $\Delta C_{SILT+CLAY}$ were not explained by any variables. Mean SIR was significantly and positively correlated to mean RER_{OLD} and RLP_{OLD}, root biomass and hemicelluloses + water-soluble compounds (Table S3, Fig. 6a,b,c,e), but negatively correlated with

mean lignin and C:N ratio (Table S3a, Figures 6d,f). Mean hemicelluloses + water-soluble compounds were significantly and negatively correlated with mean lignin content and C:N ratio (Table S3a).

When considering Pearson's correlations at the individual level (n = 36), significant correlations were found only between absorptive root diameter and ΔC_{POM}, SIR and ΔC_{SILT} (Table S3b). Correlations between root traits and mean SIR were similar compared to correlatins of raw data (Table S3b). The data dispersion in linear models showed that at the individual level, even if R^2 was low, the tendency remained the same as that when mean data were used for ΔC_{POM} (Fig. S9), ΔC_{SILT} (Fig. S10), and SIR (Fig. S11).

DISCUSSION

Total C accumulation in soil did not differ among plant species (Fig. 1), but as expected, C accumulation was significantly greater in the C_{SILT} pool beneath N_2-fixing Fabaceae, whereas in soil beneath non N_2-fixing Poaceae species, C accumulation was greater in the C_{POM} pool (Fig. 2). In line with our hypotheses, the accumulation of C into different soil C fractions, specifically C_{POM} and C_{SILT}, was correlated with root traits (Fig. 4, 5, 6). The more rapid RER and greater RLP of older roots promoted C accumulation into the C_{SILT} pool, but smaller root diameter and low content of labile compounds (hemicelluloses and water soluble compounds) enhanced C accumulation into the C_{POM} pool. Although measuring total soil organic carbon can be an easy method to evaluate C storage, it is not as sensitive to short-term C dynamics or effect of plant species and families, as the C changes in different soil fractions. Studies of C sequestration should therefore focus on better estimating C input into different C pools associated with soil textural fractions (Wiesmeier et al. 2019).

Hypothesis 1: Root elongation rate and root length production are expected to favour carbon accumulation in the C_{SILT} and $C_{SILT+CLAY}$ fractions

We hypothesized that a fast RER would promote C accumulation in coarse silt and fine silt+clay soil fractions, through an increase in exudation and microbial activity along newly initiated roots. Interestingly, RER_{OLD} and RLP_{OLD} were significantly and positively correlated with soil microbial SIR and ΔC_{SILT} (Fig. 5a,b, Table S3a), but not with the RER and RLP of newly initiated roots, that had very high rates of growth. Dennis et al. (2010) hypothesized that rapidly elongating root tips grow quickly out of the main zone of microbial activity, that is established once root exudates have been consumed. These microbial communities then consume rhizodeposits from mucilage and cell senescence as well as exudates from roots growing in proximity. Therefore, slow growing older roots would be maintained

in this zone of high microbial activity, and C accumulation in the coarse silt fraction would be higher, especially in N_2-fixing species with populations of bacteria distributed in nodules all along roots. N_2-fixing *Rhizobium* bacteria also increase root elongation (Garrido-Oter et al. 2018), likely inducing a feedback mechanism whereby a stimulated RER results in a higher exudation rate (Garcia et al. 2001), acting as a substrate for newly colonizing *Rhizobium* communities. Although the role of microbial communities is of utmost importance for C input into the soil, differences in the use of C within plants could also explain the lack of a relationship between RER_{NEW}, RLP_{NEW}, SIR and C_{SILT}. In fast-growing, newly initiated roots, we suggest that C in the form of non-structural carbohydrates (NSC, produced during photosynthesis), will be used preferentially for cell production and expansion, as found in a recent seasonal study of root elongation and NSC fluxes (Wang et al. 2018). In older roots with lower RER, less NSC is required for growth, and excess NSC would be freely exuded, reflected in the high SIR that we observed.

Contrary to our hypothesis, RER and RLP did not promote accumulation of C in the $C_{SILT+CLAY}$ pool. Surprisingly, the $C_{SILT+CLAY}$ pool was the only pool where C was actually lost over the 37 weeks, in both bare soil and beneath all plant species, and this mineralisation of C could not be explained by microbial activity or by any root traits. When soil was prepared in our study, its excavation, crushing and sieving would have disrupted soil aggregates (Franzluebbers, 1999). This increase in C mineralisation is higher in clays, as organic matter that was highly protected within the clay fraction will be released during disruption, providing a new pool of C available to microorganisms (Hassink, 1992). The presence of plant roots can also lead to an increased mineralization and loss of preexistent soil C due to an increased microbial activity (positive priming effect; Kuzyakov et al., 2000). In our study, the origin of C was not assessed, so it was not possible to quantify any priming effect. The decrease in C was mainly observed in the silt+clay fraction, challenging the assumption that the C pool associated to this soil fraction has greater C stability (Torn et al., 2009). However, these results are in line with the findings from Keiluweit et al. (2015) who observed a major priming effect in the organomineral associated C after the input of oxalic acid, a common component of root exudates. The soil disturbance, together with the input of highly degradable C, may well increase the release and priming of C in the organomineral associated fraction, thereby decreasing the C content in the silt+clay fraction, but further studies are required to better understand this phenomenon.

Hypothesis 2: more recalcitrant root traits are expected to favour the unprotected coarse POM fraction

Root traits linked to recalcitrance (high cellulose and lignin content and high C:N ratio) did not correlate to C accumulation in the coarse POM fraction (C_{POM}) (Fig. 4), but the PCA showed that this suite of traits

was sharing similar coordinates with C_{POM} on axis 1 (Fig. 3). This result is mainly due to the Poaceae species that all have higher C accumulation in the C_{POM} pool, as well as recalcitrant root traits, compared to species from other families. Recalcitrant compounds have all been reported to decrease root decomposition rates (Silver and Miya, 2001; Aulen et al. 2012; Poirier et al. 2018). Lignin-carbohydrate complexes prevent polymer-hydrolyzing enzymes access to substrates, thus reducing the degradability of plant organic matter (Cornu et al. 1994, Malherbe and Cloete, 2002). SIR, as a proxy for microbial activity, was also strongly and negatively correlated to lignin content (Fig. 6f), probably because lignin reduces the accessibility of polysaccharides to microorganisms through the formation of links between lignin and polysaccharides (Bertrand et al. 2006). Products of lignin degradation can also react with ammonia or amino acids to form further recalcitrant complexes that are less available to microorganisms (Nömmik and Vahtras, 1982). The trend observed in the PCA (Fig. 3), that species with recalcitrant tissues were linked to higher C_{POM} accumulation, is in contradiction with the lack of significant correlations between ΔC_{POM} and lignin or C:N ratio. One reason for this lack of correlation might be that the experiment was shorter than the root life span of some or all species, and full accumulation of C in the C_{POM} pool had not yet occurred (Van der Krift et Berendse, 2002). Another reason may be because C_{POM} was derived from the input of fresh C from plants, as well as losses of older C that already existed in soil. While the accumulation of new C in this fraction is influenced by the chemical composition of the root system, the losses of older, pre-existing C are not. A C labeling approach would be helpful to assess the different fluxes of new and old C and to better explain the relationships between root traits and C storage.

Interestingly, C accumulation in the C_{SILT} pool was negatively correlated with recalcitrant root traits (lignin and C:N ratio, Fig. 5e,g), but positively with hemicelluloses content and root diameter (Fig. 5d,f). Hemicelluloses comprise polysaccharides soluble in alkali and are easily degradable by microorganisms (Dekker, 1985). Hemicelluloses are usually produced to the detriment of lignin and enhance tissue degradability through higher accessibility to amorphous phases in the lignocellulose structure (Malherbe and Cloete, 2002). Microorganisms will use this easily degradable C for growth and respiration and then produce exudates and exopolysaccharides, that are used as a substrate for subsequent microbial communities (Dennis et al. 2010). These exopolysaccharides and low molecular weight compounds are belived to be the main precursors of C in the coarse silt pool (Simpson et al., 2007; Mambelli et al., 2011; Cotrufo et al. 2013, Vidal et al. 2018), probably explaining the high C_{SILT} we found beneath N_2-fixing species (Fig. 2) with high hemicelluloses + water soluble compounds and low lignin contents. Absorptive roots were negatively correlated with C accumulation in the C_{POM} pool (Fig. 4a) and positively correlated with C accumulation in the C_{SILT} pool (Fig. 5d). Absorptive roots

generally have a higher turnover rate and undergo rapid transformation through microbial degradation (McCormack et al., 2015), explaining the low accumulation in the C_{POM} pool and the positive correlation with the C_{SILT} pool. However, this relationship may also be an artefact because Poaceae roots are inherently very fine compared to Fabaceae roots (Roumet et al. 2006; 2016), highlighting that the understanding of relationships between C accumulation and morphological traits is challenging because of their inherent nature and plasticity (Poirier et al. 2018).

Hypothesis 3: Fabaceae and Poaceae strongly differ in their influence on accumulation of C into different soil fractions

Contrary to that observed in previous studies (Binkley 2005; Fornara and Tilman, 2008; Plaza-Bonilla et al., 2016; King et al., 2018), we did not find evidence of a greater accumulation of total C (ΔC_{SUM}) in soil beneath N_2-fixing species, because variability was high within Fabaceae. However, we showed that N_2-fixing species and non N_2-fixing species (especially Poaceae and Fabaceae) strongly differed in their effect on the accumulation of C into different soil C pools. Roots of Poaceae, as compared to Fabaceae, had a lower RER and RLP. Poaceae produced thinner roots, rich in lignin and cellulose with a high C:N ratio. These more recalcitrant tissues slow down microbial activity and hence root decomposition rate (Roumet et al. 2016; Freschet et al. 2017). Due to their particular chemical composition, non N_2-fixing species, especially Poaceae species, promote C accumulation in the unprotected C_{POM} pool, and have a lower C accumulation in the more stable C_{SILT} pool (Fig. 4). On the other hand, roots of N_2-fixing Fabaceae grow faster and produce thick roots that are easily degradable, since they are rich in N (low C:N ratio) and in hemicelluloses and water soluble compounds. These traits favour the development of microbial biomass and enhance their activity, as observed from the SIR that was 40% higher beneath Fabaceae than Poaceae species (see Fig. 7 for a conceptual model describing C accumulation in different pools).

Because of the lack of correlations between root traits and C accumulation in $C_{SILT+CLAY}$ pool, we cannot establish that the $C_{SILT+CLAY}$ pool increases when there is a higher input of labile C from N_2-fixing species. C accumulation is the result of the input of new C and losses of pre-existing C that can be influenced by the input of fresh C from plants (priming effect). The difference in behavior between these two pools could result in poor correlations between root traits and C accumulation in the $C_{SILT+CLAY}$ pool. An isotopic approach differentiating between the changes in new C and old C in different C pools, allowing us to assess the priming effect, would be fundamental to understanding the mechanisms behind soil C storage (Rossi 2019).

CONCLUSION

Our findings show that specific plant root traits influence the accumulation of C into different pools, largely through the mediation of microbial activity, shaping the C pathway in soil and, finally, its persistence. Our results showed that non N_2-fixing Poaceae species, characterized by high contents of lignin and cellulose and a high C:N, promoted accumulation of C in the unprotected coarse POM fraction, while root traits associated with high labile C input (high hemicelluloses + water soluble compound contents, high RER_{OLD} and RLP_{OLD}) and microbial activity, typical of N_2-fixing Fabaceae species, stimulated C accumulation in the protected coarse silt fraction. Root elongation rate and length production promoted microbial activity in older roots only, potentially suggesting either a spatial influence of root exudate accessibility on microbial communities, or a relationship between non-structural carbohydrate use in roots and available exudates for microbial consumption. The planting of vegetation in bare soil also led to a loss of C in the fine silt+clay fraction, commonly belived to be the most stable. Differentiating the source of C loss (pre-existing C in soil or fresh C from live plants), is a fundamental step to assess the priming effect and understand the mechanisms behind C loss in the finer soil fractions, and could be achieved through an isotope labelling approach. Longer term studies on C dynamics are needed to understand these species and root trait effects over time and the consequent C accumulation in different pools. Moreover, the influence of different soils and associated microbial communities need to be taken into consideration for a broader understanding of C pool dynamics. Our results will not only be useful for identifying plant species capable of enhancing long-term C storage in soil, but will also contribute significantly to the understanding of mechanistic processes within the C cycle.

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TABLES AND FIGURES

Table 1: Plant root traits and SIR for the 12 herbaceous species. Mean data ± standard deviation are given for species from N_2-fixing Fabaceae , N_2-fixing Poaceae families, and N_2-fixing species aggregated (Poaceae species, *P. lanceolate* and *S. minor*). For each species and for N_2-fixing Fabaceae , N_2-fixing Poaceae families, and N_2-fixing species aggregated, mean and standard deviation are given. Abbreviations: RER_{TOT} – root elongation rate of the entire root system; RER_{OLD} – of old roots older than 2 weeks; RER_{NEW} – of new roots younger than 2 weeks; RLP_{TOT} root length production of the entire root system; RLP_{OLD} – of old roots; RLP_{NEW} – of new roots; Root biomass – total root biomass of a core sampled after 37 weeks; Diameter absorptive – mean diameter of absorptive roots after 37 weeks; Hemicell. + H_20 – concentration of hemicelluloses and water soluble compounds in absorptive roots; Cellulose, Lignin – concentrations of cellulose and lignin in absorptive roots; C:N – ratio of carbon and nitrogen in absorptive roots; SIR – microbial subsrate induced respiration. Different letters next to the average value indicate statistically significant differences ($p < 0.05$) between species or families according to Tukey HSD tests. DF – degree of freedom (number of species - 1, number of observations). Statistically significant values ($p < 0.05$) are indicated in bold text.

Family	**Fabaceae**					**Poaceae**					**Rosaceae**	**Plantaginaceae**	**Effect of species (ANOVA)**			**N_2-fixing (Fabaceae)**	**Non N_2-fixing (Only Poaceae)**	**Effect of families (ANOVA) Poaceae VS Fabceae**			**Non N_2-fixing (All: Poaceae, *S. minor, P. lanceolata*)**	**Effect of N aquisition strategy (ANOVA) N_2-fixing VS non N_2fixing**		
Species	*Lotus corniculatus*	*Trifolium repens*	*Trifolium pratense*	*Onobrychis viciifolia*	*Medicago sativa*	*Bromus erectus*	*Festuca rubra*	*Dactylis glomerata*	*Poa pratensis*	*Lolium perenne*	*Sanguisorba minor*	*Plantago lanceolata*												
Acronym code	Lc	Tr	Tp	Ov	Ms	Be	Fr	Dg	Pp	Lp	Sm	Pl	Df	F	*p*			Df	F	*p*		Df	F	*p*
RER_{TOT} (mm d^{-1})	0.55±1.1 (a)	0.75±1.5 (a)	0.59±1 (a)	0.53±10 (a)	0.57±0.9 (a)	0.42±0.6 (a)	0.23±0.50 (a)	0.58±0.9 (a)	0.66±2.90 (a)	0.42±0.8 (a)	0.39±0.5 (a)	0.5±0.7 (a)	11,175	18.9	0.061	0.57±0.08	0.42±0.13	2,9	1.8	0.22	0.46±0.14	1,10	3.94	0.075
RER_{OLD} (mm d^{-1})	0.17±0.07 (a)	0.2±0.08 (a)	0.4±0.11 (a)	0.21±0.05 (a)	0.26±0.08 (a)	0.12±0.03 (a)	0.09±0.04 (a)	0.14±0.06 (a)	0.05±0.02 (a)	0.11±0.04 (a)	0.13±0.04 (a)	0.16±0.04 (a)	11,178	17.9	0.081	0.25±0.09	0.13±0.03	2,9	6.3	**0.02**	0.12±0.04	1,11	12.46	**0.005**
RER_{NEW} (mm d^{-1})	0.99±0.27 (a)	0.91±0.21 (a)	0.73±0.15 (a)	0.81±0.16 (a)	0.97±0.12 (a)	1.06±0.29 (a)	0.32±0.08 (a)	1.13±0.25 (a)	0.96±0.31 (a)	0.67±0.11 (a)	0.66±0.08 (a)	0.76±0.11 (a)	11,178	17.4	0.11	0.88±0.11	0.79±0.33	2,9	0.37	0.7	0.79±0.28	1,12	0.425	0.529
RLP_{TOT} (m)	3.03	3.26	3.36	3.62	3.61	2.55	1.19	2.76	2.16	2.95	2.31	3.04	-	-	-	3.37±2.32	2.32±0.7	2,9	5.17	**0.03**	2.42±0.63	1,13	10.05	**0.009**
RLP_{OLD} (m)	4.51	4.91	4.26	4.96	5.23	3.47	1.05	3.3	2.76	4.29	3.32	4.5	-	-	-	1.33±0.61	0.61±0.2	2,9	7.5	**0.01**	0.65±0.23	1,14	16.05	**0.003**
RLP_{NEW} (m)	0.89	1.05	1.78	1.35	1.6	0.78	0.53	0.81	0.32	0.62	0.47	0.99	-	-	-	4.78±2.97	2.97±1.21	2,9	5.12	**0.03**	3.24±1.14	1,15	8.181	**0.02**
Root biomass (g)	1.53±2.6 (bc)	0.55±1.13 (c)	2.01±0.62 (b)	2.06±1.1 (b)	4.23±0.42 (a)	0.65±0.08 (c)	0.70±1.6 (c)	0.58±0.51 (c)	0.60±0.83 (c)	0.57±0.57 (c)	0.91±2.13 (c)	0.49±0.60 (c)	11,24	27.3	**<0.001**	2.08±1.33	0.62±0.11	1,28	10.9	**<0.001**[1]	0.65±0.17	1,31	19.3	**<0.001**[1]
Diameter absorptive roots (mm)	0.47±0.05 (b)	0.28±0.05 (de)	0.32±0.04 (cd)	0.55±0.18 (a)	0.35±0.18 (c)	0.27±0.02 (def)	0.22±0.05 (fg)	0.21±0.14 (g)	0.22±0.19 (g)	0.22±0.05 (fg)	0.26±0.09 (efg)	0.28±0.14 (de)	11,23	98.7	**<0.001**	0.39±0.11	0.23±0.03	1,27	31.7	**<0.001**	0.25±0.03	1,31	33.5	**<0.001**
Hemicell. +H_2O soluble compounds (mg g^{-1})	779.5±58.1 (a)	612±8.3 (bcde)	674.5±44.6 (abc)	704.1±78.8 (ab)	755.1±31 (a)	533.9±12.1 (de)	572.3±27.3 (cde)	530.4±57.6 (de)	562.4±31.2 (cde)	520.5±31.7 (e)	703.2±NA (ab)	639.4±12.7 (bcd)	11,21	17.4	**<0.001**	705.11±74.39	543.51±33.56	1,25	51.5	**<0.001**	583.86±69.51	1,31	23.11	**<0.001**
Cellulose (mg g-1)	101.6±6.3 (a)	163.2±8.7 (a)	102.5±17.7 (a)	120±69.3 (a)	123.9±27.7 (a)	177.9±76.5 (a)	160.7±44.8 (a)	137.5±7.7 (a)	89.1±6 (a)	181.6±32.3 (a)	140±NA (a)	151.2±54 (a)	11,21	1.7	0.13	122.39±33.61	154.89±50.8	1,25	3.9	0.06	151.96±45.55	1,31	4.2	**0.049**
Lignin (mg g-1)	118.9±57.8 (c)	224.8±0.4 (abc)	223±33.7 (abc)	175.9±9.4 (abc)	120.9±3.5 (c)	288.3±88.5 (ab)	267±39.5 (ab)	332.1±65.3 (a)	348.4±25.1 (a)	297.9±62.1 (ab)	156.8±NA (bc)	209.3±66.7 (abc)	11,21	6.7	**<0.001**	172.5±56.53	301.6±59.02	1,25	33.7	**<0.001**	264.18±79.06	1,31	13.63	**<0.001**
C:N ratio	15.4±0.8 (c)	21.2±1.1 (c)	17.6±3.20 (c)	20.5±3.2 (c)	21.4±2 (c)	50.8±7.6 (b)	61.3±2.6 (ab)	62.5±6.4 (ab)	59.1±4.1 (ab)	61±4.4 (ab)	69.8±3.2 (a)	68.9±3.1 (a)	11,21	96.4	**<0.001**	19.15±3.07	58.67±6.34	1,25	436	**<0.001**	62.04±7.41	1,31	413.4	**<0.001**
SIR (µg C-CO_2 g^{-1} soil h^{-1})	5.37±0.46 (ab)	4.43±0.48 (bc)	6.11±0.35 (a)	4.07±0.18 (bcd)	6.41±0.56 (a)	2.47±0.34 (d)	3.22±0.11 (cd)	3.58±0.28 (cd)	3.15±0.15 (cd)	3.17±0.1 (cd)	3.16±0.23 (cd)	2.99±0.11 (cd)	9,20	16	**<0.001**	5.28±1	3.12±0.4	1,28	45.9	**<0.001**	3.1±0.46	1,31	60.05	**<0.001**

836 **Fig.1:** Comparison of the difference ($\Delta C = C_{t37} - C_{t0}$) in carbon (C) after 37 weeks between different soil fractions for each species. a) total C (ΔC_{SUM}), b) C in the coarse POM fraction (ΔC_{POM}), c) C in the fine POM fraction ($\Delta C_{finePOM}$), d) C in
837 the coarse silt fraction (ΔC_{SILT}) and e) C in the fine silt+clay fraction ($\Delta C_{SILT+CLAY}$). In each boxplot, the lower edge of the box corresponds to the 25th percentile data point, while the top edge of the box corresponds to the 75th percentile
838 data point. The line within the box represents the median.

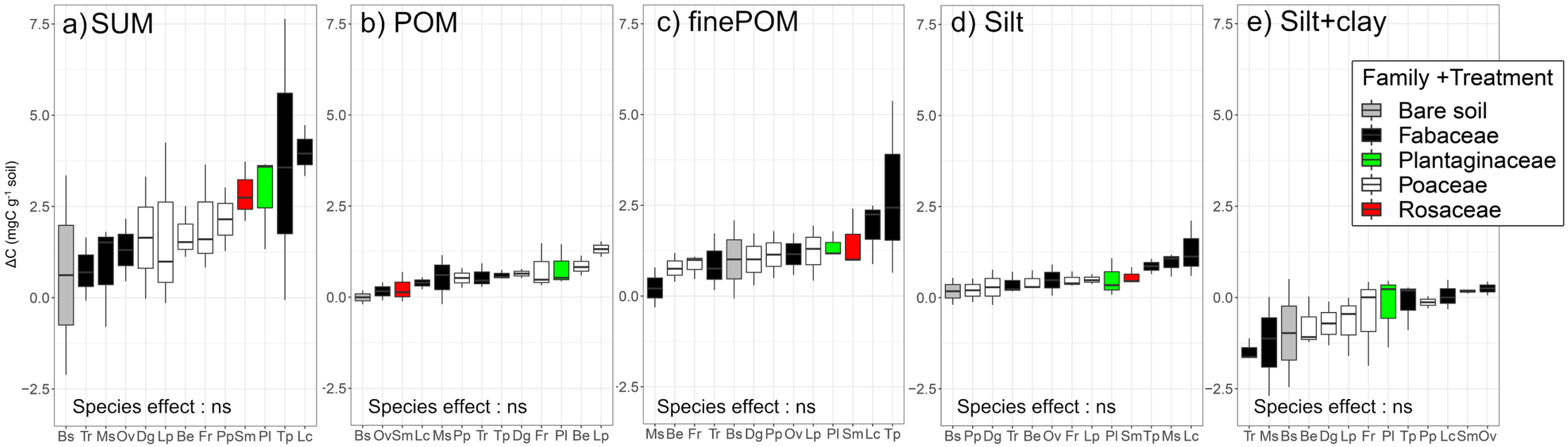

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Fig.2: Comparison of the difference ($\Delta C = C_{t37} - C_{t0}$) in carbon (C) after 37 weeks among N_2-fixing Fabace, non N_2-fixing Poaceae species only and non N_2-fixing species aggregated (Poaceae, P. lanceolate, S. Minor), and control.a) C in the coarse POM fraction (ΔC_{POM}) and b) C in the silt fraction (ΔC_{silt}). No significant differences were found in total C (ΔC_{SUM}), C in the fine POM fraction ($\Delta C_{finePOM}$), or in C in the silt+clay fraction ($\Delta C_{silt+clay}$). In each boxplot, the lower edge of the box corresponds to the 25th percentile data point, while the top edge of the box corresponds to the 75th percentile data point. The line within the box represents the median and black dots indicate outliers. Different letters above the boxplots indicate statistically significant differences ($p < 0.05$) among families and control according to a Tukey HSD test. The blue leter refer to the Tuckey HSD test perfomed between control, N_2-fixing Fabaceae species and N_2-fixing Poaceae species only, while the red letters refer to the Tuckey HSD test performed between control, N_2-fixing Fabaceae species and N_2-fixing species aggregated together.Note the graduation differences in *y*-axis between a) and b).

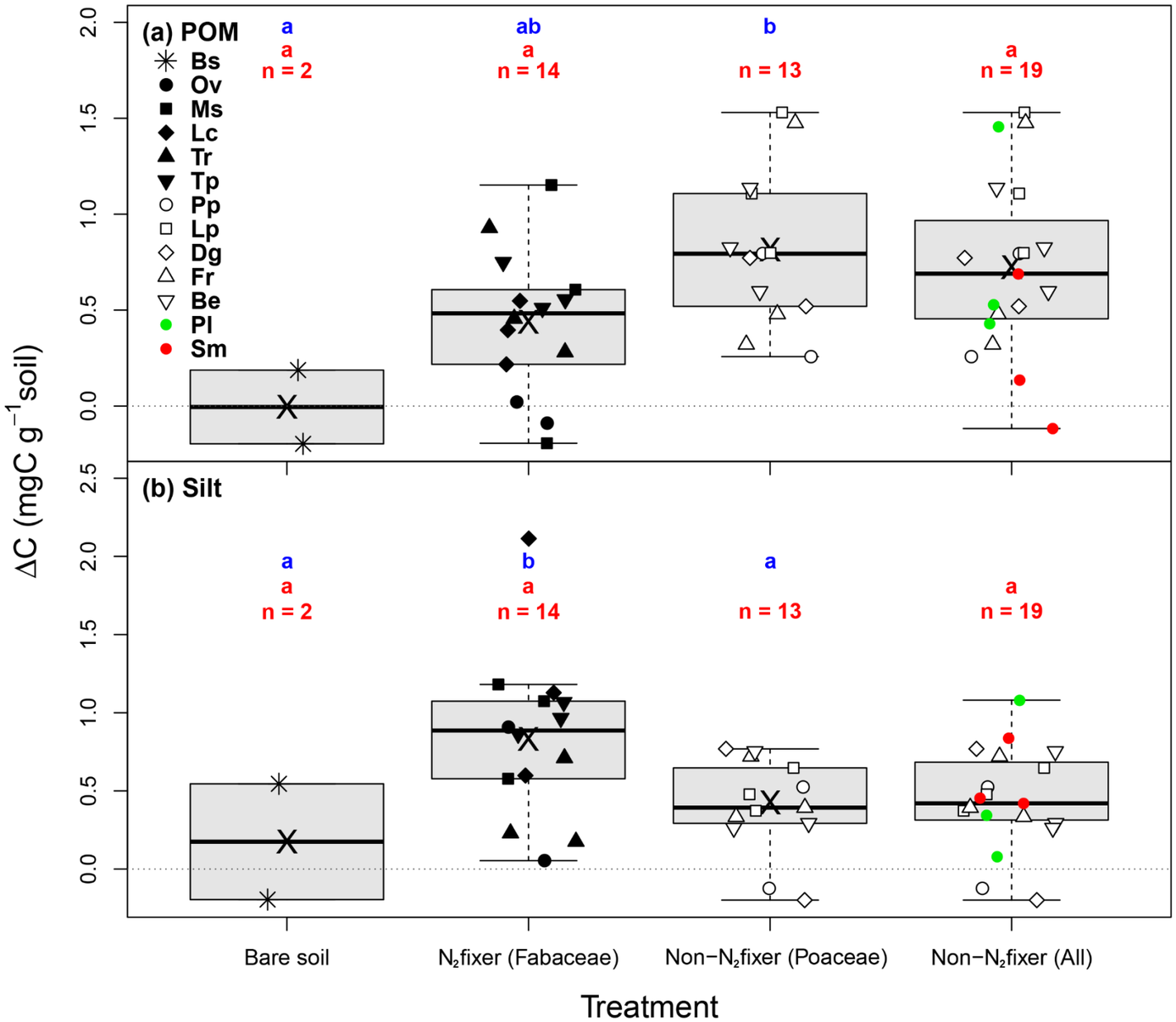

Fig.3: Principal Component Analysis of six soil variables (five carbon pool changes and SIR) and six root variables measured on 12 species. Black dots are Fabaceae, white dots are Poaceae, red dots are *Sanguisorba minor*, and green dots are *Plantago lanceolata*. The Hull polygons unify the different replicates for the same species. Abbreviations: SIR – microbial subsrate induced respiration; Hemicelluloses + H_20 – concentration of hemicelluloses and water soluble compounds in absorptive roots, cellulose, lignin – concentrations of cellulose and lignin in absorptive roots; C:N – ratio of carbon and nitrogen in absorptive roots; ΔC_{POM} – difference ($\Delta C = C_{t37} - C_{t0}$) in carbon (C) after 37 weeks for the coarse POM C pool; $\Delta C_{finePOM}$ – for the fine POM C pool; ΔC_{SILT} – for the silt C pool; $\Delta C_{SILT+CLAY}$ – for the silt + clay f C pool; ΔC_{SUM} – sum of different fractions, ΔC as the total change in C concentration in soil.

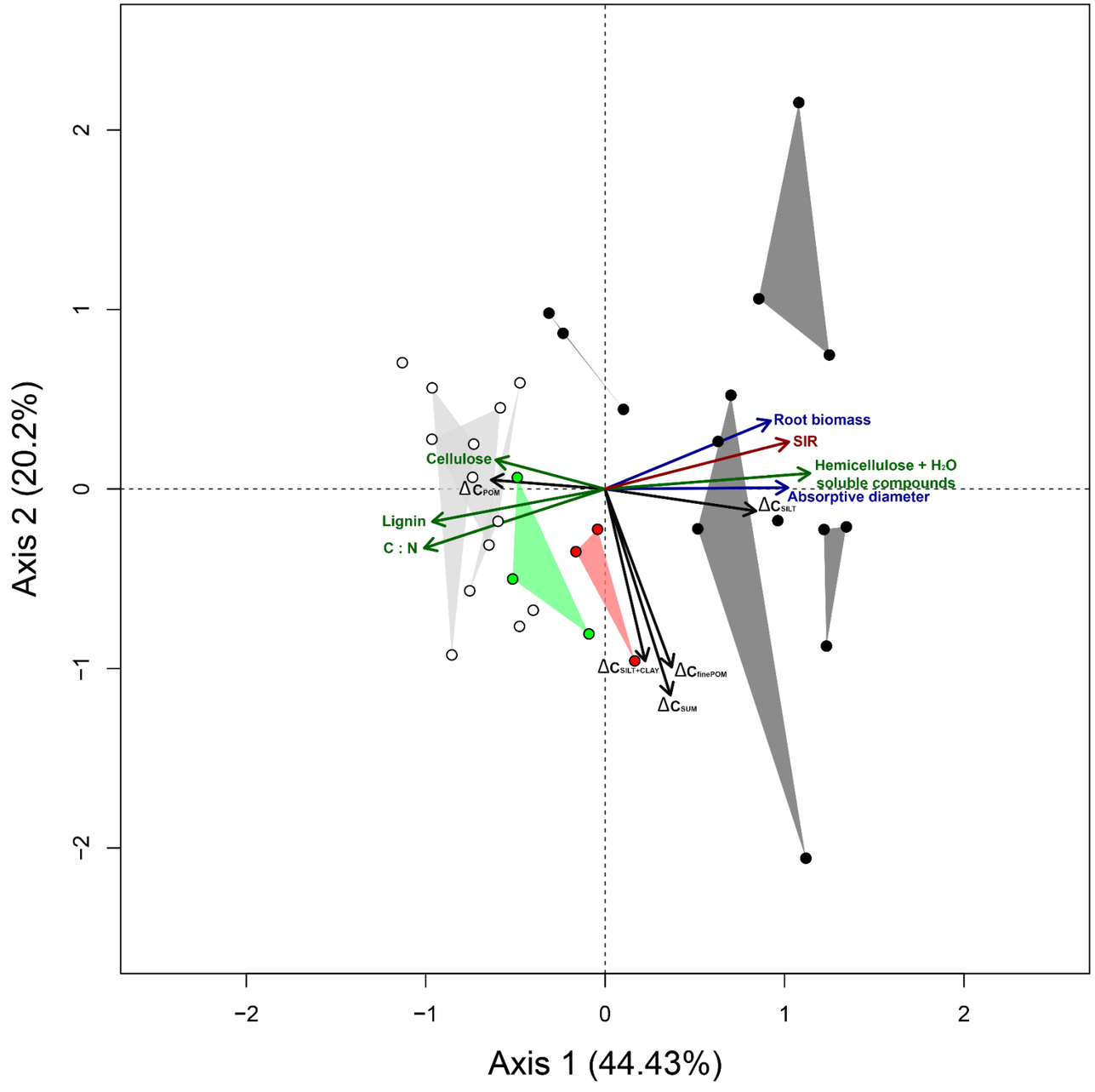

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Fig. 4: Linear regression at species level (n=12) between ΔC_{POM} as the difference ($\Delta C = C_{t37} - C_{t0}$) in carbon (C) after 37 weeks for the coarse POM C pool and a) diameter of absorptive roots and b) hemicelluloses + water soluble compounds. The black symbols are the N_2-fixing Fabaceae species, the white symbols the non N_2-fixing Poaceae species, the red dots are *S. minor* and the green dots are *P. lanceolata*. The red line is the linear model function of the variables and R^2, F and *p* of the linear model are shown.

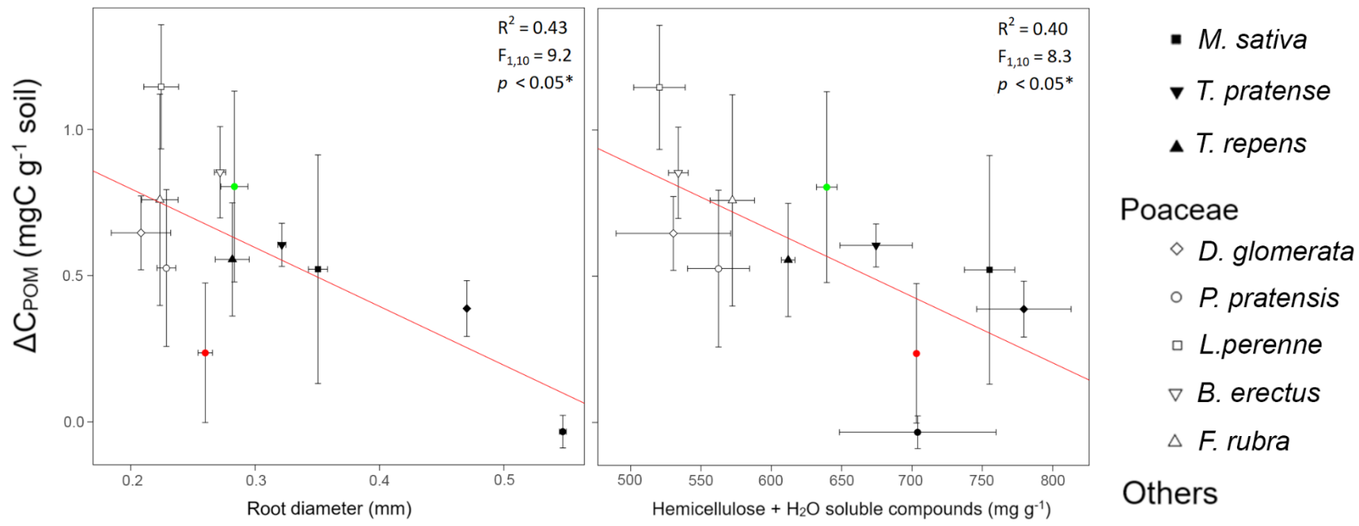

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Fig. 5: Linear regression at species level (n=12) between ΔC_{silt} as difference ($\Delta C = C_{t37} - C_{t0}$) in carbon (C) after 37 weeks for the coarse silt C pool and a) root elongation rate of old roots (RER_{OLD}), b) root length production of old roots (RLP_{OLD}), c) root biomass, d) diameter of absorptive roots, e) lignin content, f) hemicelluloses + water soluble compounds, g) C:N ratio and h) substrate induced respiration rate (SIR). The black symbols are the N_2-fixing Fabaceae species, the white symbols the non N_2-fixing Poaceae species, the red dots are *S. minor* and the green dots are *P. lanceolata*. The red line is the linear model function of the variables and R^2, F and *p* of the linear model are shown.

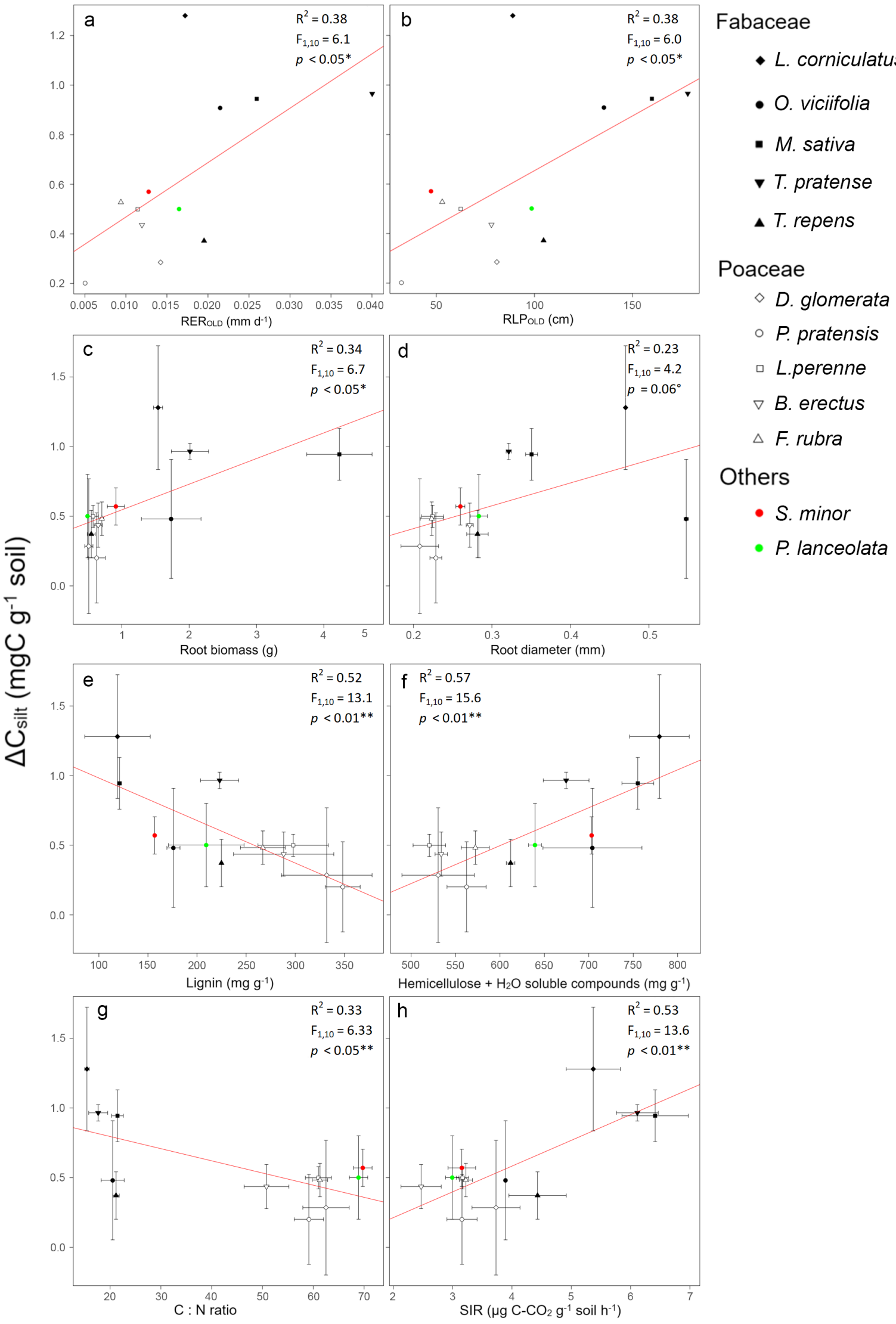

Fig. 6: Linear regression at species level (n=12) between substrate induced respiration rate (SIR) and a) root elongation rate of old roots (RER_{OLD}), b) root length production of old roots (RLP_{OLD}), c) root biomass, d) lignin content, e) hemicelluloses + water soluble compounds, f) C:N ratio. The black symbols are the N_2-fixing Fabaceae species, the white symbols the non N_2-fixing Poaceae species, the red dots *S. minor* and the green dots *P. lanceolata*. The red line is the linear model function of the variables and R^2, F and *p* of the linear model are shown.

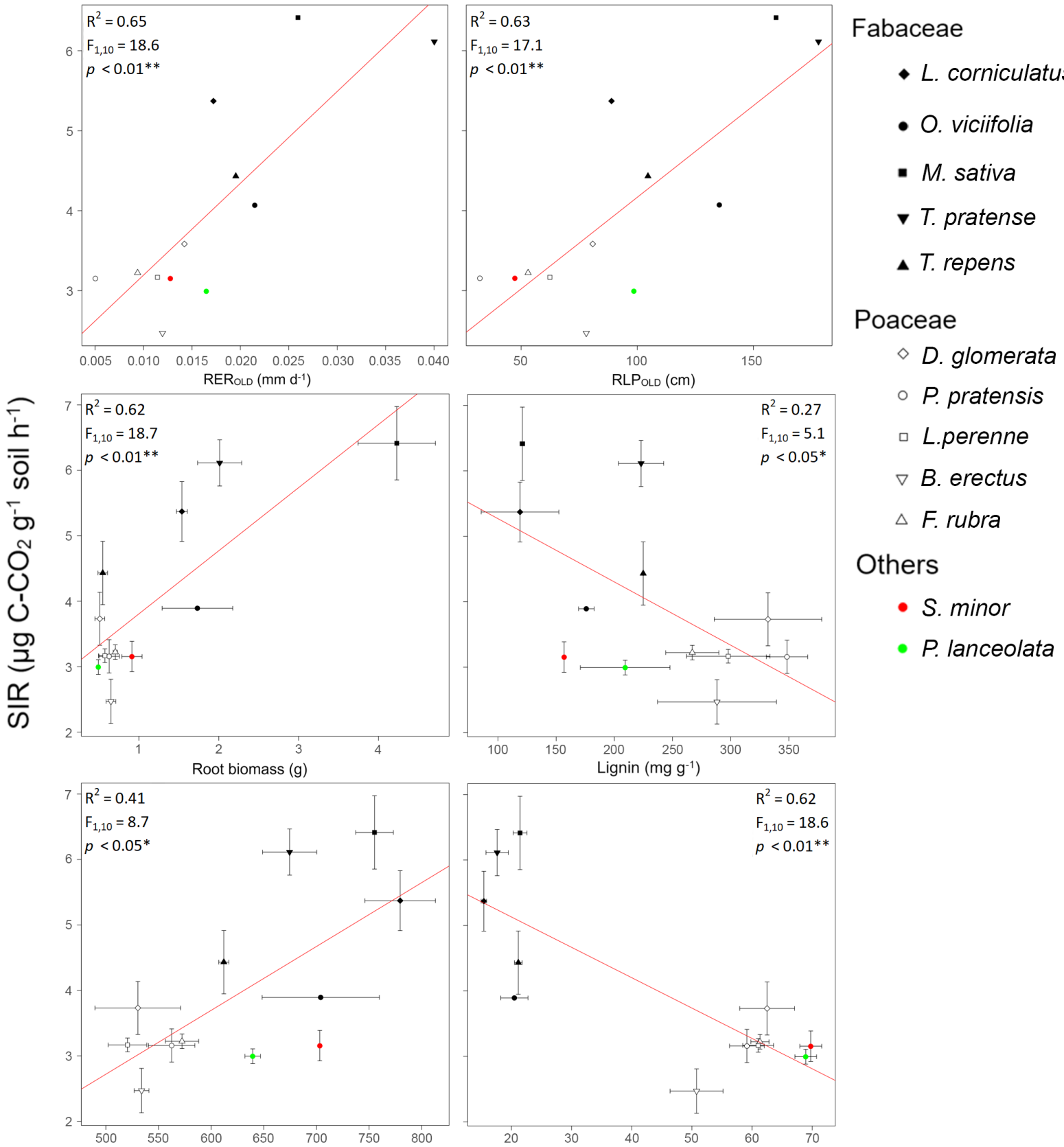

Fig.7: Conceptual scheme of carbon (C) sequestration mechanisms into different soil pools under N_2-fixing (Fabaceae) and non N_2-fixing (Poaceae) species. Square boxes refer to the major factors affecting C sequestration. Ellipses show the destination of C into unprotected pools (POM and finePOM) and protected pools (coarse silt and fine silt+clay). Text in the central column describes the mechanisms favoring C sequestration into soil C pools. The arrows symbolize the processes of transformation or transport of C into different pools. Arrow colors represent the nature of the C: red C derived from root turnover, green C from rhizodeposition, yellow C from microbial exudates and exopolysaccharides and blue the C respired back into the atmosphere as CO_2. The thickness of the arrows is qualitative, with wider arrows reflecting higher C fluxes. The signs: "+" (in green) means an increase and "–" (in red) means a decrease.