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

Lorenzo Matteo Walter Rossi, Zhun Mao, Luis Merino-Martin, Catherine Roumet, Florian Fort, Olivier Taugourdeau, Hassan Boukcim, Stéphane Fourtier, Maria del Rey Granado, Tiphaine Chevallier, et al.

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1 **Supplementary Materials**

2 **Article: Pathways to persistence: plant root traits alter carbon accumulation in different soil carbon**
3 **pools**

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23 **Table S1:** Pearson's correlation coefficients (r) showing relationships between mean root elongation rate and climatic variables.

24 Variables tested include: mean daily soil temperature, mean daily air temperature, minimum daily air temperature, maximum daily air temperature, mean
 25 daily solar radiation and a) RER_{TOT}: mean root elongation rate of a single root, b) RER_{OLD}: mean root elongation rate of a single root that was already present
 26 at the previous sampling date (i.e. that were older than 14 days), c) RER_{NEW}: mean root elongation rate of a single newly initiated root (i.e. that were aged 1
 27 to 14 days). The correlations were performed for data from each RER sampling date, i.e. every 2 weeks for each species over the 10 month period.

a - Correlation between climate factors and RER _{TOT} of the total root system							b - Correlation between climate factors and RER _{OLD} of the old roots						
		Soil temperature	Mean air temperature	Minimum air temperature	Maximum air temperature	Mean solar radiation	Family	Species	Soil temperature	Mean air temperature	Minimum air temperature	Maximum air temperature	Mean solar radiation
Poaceae	<i>Dactylis glomerata</i>	-0.23	-0.21	-0.1	-0.18	-0.1	Poaceae	<i>Dactylis glomerata</i>	-0.27	-0.26	-0.17	-0.22	-0.12
	<i>Lolium perenne</i>	0.01	-0.04	-0.03	0.09	0.38		<i>Lolium perenne</i>	0.14	0.13	0.31	0.09	0.21
	<i>Festuca rubra</i>	-0.21	-0.27	-0.34	-0.2	0.15		<i>Festuca rubra</i>	-0.25	-0.3	-0.41	-0.25	0.05
	<i>Bromus erectus</i>	-0.02	-0.05	-0.01	0.02	0.29		<i>Bromus erectus</i>	0.17	0.28	-0.07	0.2	0.29
	<i>Poa pratensis</i>	0.1	0.08	0.34	0.1	0.43		<i>Poa pratensis</i>	-0.03	0	0.4	0.04	0.21
Fabaceae	<i>Trifolium repens</i>	0.76***	0.81***	0.45	0.68**	0.33	Fabaceae	<i>Trifolium repens</i>	0.28	0.28	0.15	0.4	0.45
	<i>Trifolium pratense</i>	0.02	-0.02	-0.02	0.09	0.4		<i>Trifolium pratense</i>	0.18	0.25	-0.07	0.24	0.38
	<i>Lotus corniculatus</i>	0.70**	0.70**	0.44	0.68**	0.72**		<i>Lotus corniculatus</i>	0.73**	0.71**	0.51	0.74**	0.89***
	<i>Onobrychis viciifolia</i>	0.13	0.09	0.3	0.17	0.54*		<i>Onobrychis viciifolia</i>	0.04	0.02	0.26	0.07	0.36
	<i>Medicago sativa</i>	0.01	0.01	-0.05	0.02	0.22		<i>Medicago sativa</i>	0.03	0.01	-0.05	0.03	0.23
Rosaceae	<i>Sanguisorba minor</i>	-0.06	-0.06	-0.06	-0.04	0.16	Rosaceae	<i>Sanguisorba minor</i>	0.13	0.2	-0.08	0.1	-0.33
Plantaginaceae	<i>Plantago lanceolata</i>	0	-0.04	0	0.04	0.3	Plantaginaceae	<i>Plantago lanceolata</i>	-0.05	-0.07	-0.05	-0.03	0.21

c - Correlation between climate factors and RER _{NEW} of new roots						
		Soil temperature	Mean air temperature	Minimum air temperature	Maximum air temperature	Mean solar radiation
Poaceae	<i>Dactylis glomerata</i>	-0.55*	-0.54*	-0.47	-0.60*	-0.66**
	<i>Lolium perenne</i>	0.03	0	-0.05	0.1	0.29
	<i>Festuca rubra</i>	-0.11	-0.16	-0.04	-0.15	0.26
	<i>Bromus erectus</i>	-0.06	-0.07	-0.08	-0.05	0.17
	<i>Poa pratensis</i>	0.09	0.08	0.36	0.06	0.38
Fabaceae	<i>Trifolium repens</i>	0.59*	0.67**	0.17	0.57*	0.17
	<i>Trifolium pratense</i>	0.11	0.1	0.37	0.08	0.41
	<i>Lotus corniculatus</i>	0.76***	0.75**	0.48	0.76***	0.76**
	<i>Onobrychis viciifolia</i>	0.13	0.1	0.3	0.15	0.51
	<i>Medicago sativa</i>	0.04	0.06	-0.35	0.04	0.21
Rosaceae	<i>Sanguisorba minor</i>	0.09	0.16	0.04	0.06	-0.05
Plantaginaceae	<i>Plantago lanceolata</i>	-0.05	-0.06	-0.06	-0.03	0.19

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32 **Table S2:** Pearson's correlation coefficients (r) showing relationships between cumulative root length production and climatic variables.

33 Variables tested include: mean daily soil temperature, mean daily air temperature, minimum daily air temperature, maximum daily air temperature, mean
 34 daily solar radiation and a) RLP_{TOT}: cumulative root length production of the 30 roots that were analysed, b) RLP_{OLD}: cumulative root length production of roots
 35 that were already present at the previous sampling date (i.e. that were older than 14 days), c) RLP_{NEW}: cumulative root length production of newly initiated
 36 roots (i.e. that were aged 1 to 14 days). The correlations were performed for data from each RLP sampling date, i.e. every 2 weeks for each species over the
 37 10 month period.

a - Correlation between climate factors and RLP _{TOT} of the total root system						
		Soil temperature	Mean air temperature	Minimum air temperature	Maximum air temperature	Average solar radiation
Poaceae	<i>Dactylis glomerata</i>	-0.4	-0.41	-0.46	-0.45	-0.38
	<i>Lolium perenne</i>	-0.02	-0.05	-0.08	0.04	0.29
	<i>Festuca rubra</i>	-0.19	-0.25	-0.32	-0.18	0.18
	<i>Bromus erectus</i>	-0.01	-0.05	0.01	0.03	0.31
	<i>Poa pratensis</i>	0.12	0.09	0.35	0.12	0.46
Fabaceae	<i>Trifolium repens</i>	0.36	0.37	-0.01	0.33	0.38
	<i>Trifolium pratense</i>	0.03	0	0.03	0.07	0.34
	<i>Lotus corniculatus</i>	0.72**	0.72**	0.46	0.70**	0.74**
	<i>Onobrychis viciifolia</i>	0.13	0.09	0.3	0.17	0.54*
	<i>Medicago sativa</i>	0.01	-0.01	-0.04	0.02	0.24
Rosaceae	<i>Sanguisorba minor</i>	-0.06	-0.07	-0.05	-0.04	0.18
Plantaginaceae	<i>Plantago lanceolata</i>	0	-0.04	0	0.04	0.31

b - Correlation between climate factors and RLP _{OLD} of the old roots						
		Soil temperature	Mean air temperature	Minimum air temperature	Maximum air temperature	Mean solar radiation
Poaceae	<i>Dactylis glomerata</i>	-0.29	-0.34	-0.38	-0.28	0
	<i>Lolium perenne</i>	0	-0.07	-0.02	-0.08	0.24
	<i>Festuca rubra</i>	-0.16	-0.22	-0.31	-0.15	0.19
	<i>Bromus erectus</i>	0.18	0.25	0	0.23	0.38
	<i>Poa pratensis</i>	0.02	0.03	0.4	0.1	0.31
Fabaceae	<i>Trifolium repens</i>	0.28	0.26	0.2	0.41	0.51*
	<i>Trifolium pratense</i>	0.22	0.26	0.02	0.29	0.47
	<i>Lotus corniculatus</i>	0.72**	0.69**	0.52	0.75****	0.90****
	<i>Onobrychis viciifolia</i>	0.06	0.03	0.28	0.1	0.42
	<i>Medicago sativa</i>	0.04	0	0.02	0.08	0.33
Rosaceae	<i>Sanguisorba minor</i>	-0.04	-0.05	-0.06	-0.02	0.18
Plantaginaceae	<i>Plantago lanceolata</i>	0	-0.04	0.02	0.05	0.33

c - Correlation between climate factors and RLP _{NEW} of new roots						
		Soil temperature	Mean air temperature	Minimum air temperature	Maximum air temperature	Mean solar radiation
Poaceae	<i>Dactylis glomerata</i>	-0.52*	-0.51*	-0.51	-0.54*	-0.48
	<i>Lolium perenne</i>	-0.25	-0.3	-0.43	-0.27	0.04
	<i>Festuca rubra</i>	-0.08	-0.14	-0.03	-0.1	0.33
	<i>Bromus erectus</i>	0.01	-0.03	0.01	0.05	0.33
	<i>Poa pratensis</i>	0.13	0.1	0.34	0.12	0.46
Fabaceae	<i>Trifolium repens</i>	0.51*	0.57*	0.11	0.49	0.26
	<i>Trifolium pratense</i>	0.17	0.14	0.36	0.17	0.51*
	<i>Lotus corniculatus</i>	0.69**	0.69**	0.41	0.70**	0.77****
	<i>Onobrychis viciifolia</i>	0.06	0.01	0.02	0.13	0.43
	<i>Medicago sativa</i>	0.03	0.02	-0.03	0.05	0.27
Rosaceae	<i>Sanguisorba minor</i>	0.17	0.14	0.19	0.22	0.46
Plantaginaceae	<i>Plantago lanceolata</i>	0.01	-0.03	0.01	0.05	0.32

38

39 **Table S3:** Pearson's correlation coefficients (*r*) showing relationships between root variables and soil variables. Table S3a) shows the significant correlations
 40 obtained with the means of the three replicates for every soil and root characteristic for a total of n=12. Table S3b) shows the significant Pearson's correlations
 41 obtained using all data where n = 34. Abbreviations: RER_{TOT} – root elongation rate of the entire root system; RER_{OLD} – of roots older than 14 days; RER_{NEW} – of
 42 new roots aged 1 – 14 days; RLP_{TOT} – root length production of the entire root system; RLP_{OLD} – of old roots; RLP_{NEW} – of new roots; Root biomass – total root
 43 biomass of a core sampled at 37 weeks; absorptive root diameter– mean diameter of absorptive roots at 37 weeks; hemicellulose + water soluble compounds
 44 – concentration of hemicellulose and water soluble compounds in absorptive roots; cellulose, lignin – concentrations of cellulose and lignin in absorptive
 45 roots; C:N – ratio of carbon to nitrogen in absorptive roots; SIR – microbial substrate induced respiration.

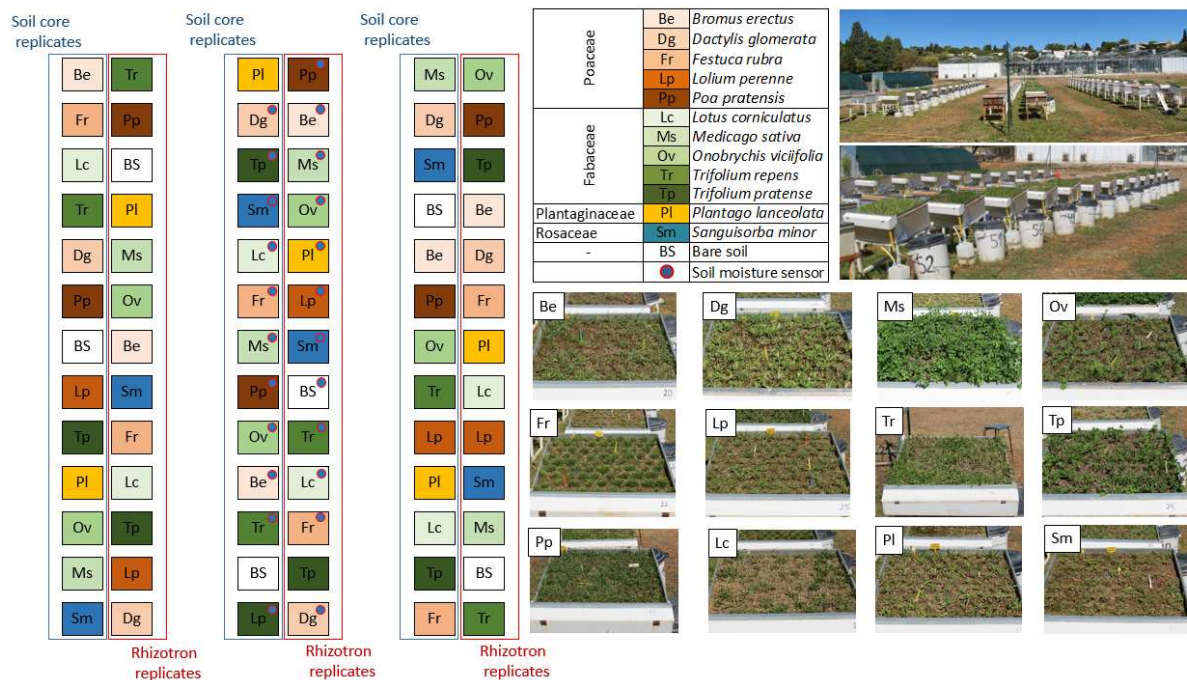
a) Pearson's correlations at species level (n = 12, mean of three replicates)								b) Pearson's correlations at individual level (n = 34)					
		ΔC_{SUM}	ΔC_{POM}	$\Delta C_{finePOM}$	ΔC_{silt}	$\Delta C_{silt+clay}$	SIR	ΔC_{SUM}	ΔC_{POM}	$\Delta C_{finePOM}$	ΔC_{silt}	$\Delta C_{silt+clay}$	SIR
Root growth dynamics	RER _{TOT}	-0.32	-0.2	-0.06	-0.1	-0.17	0.45	-0.32	-0.2	-0.06	-0.1	-0.17	0.45
	RER _{OLD}	-0.17	-0.25	-0.04	0.72**	0.37	0.74**	-0.17	-0.25	-0.04	0.72**	0.37	0.74**
	RER _{NEW}	-0.24	-0.13	-0.2	-0.12	-0.55	0.18	-0.24	-0.13	-0.2	-0.12	-0.55	0.18
	RLP _{TOT}	-0.34	-0.33	-0.18	0.37	0.34	0.51	-0.34	-0.33	-0.18	0.37	0.34	0.51
	RLP _{NEW}	-0.3	-0.3	-0.15	0.33	0.17	0.43	-0.3	-0.3	-0.15	0.33	0.17	0.43
	RLP _{OLD}	-0.32	-0.25	-0.22	0.66*	0.34	0.70*	-0.32	-0.25	-0.22	0.66*	0.34	0.70*
Root morphological traits	Root biomass	-0.2	-0.46	-0.37	0.78**	0.3	0.80**	0.18	-0.08	0.02	0.3	-0.07	0.69***
	Absorptive roots diameter	-0.2	-0.72**	-0.17	0.79**	0.14	0.56	0.08	-0.44*	0.21	0.14	0.36	0.50**
Root chemical traits	Hemicelluloses +H ₂ O soluble compounds	-0.06	-0.61*	-0.13	0.82**	-0.3	0.68*	0.26	-0.3	0.25	0.22	0.23	0.60***
	Cellulose	-0.18	0.47	-0.14	-0.22	0.19	-0.53	-0.24	0.13	-0.16	-0.03	0.18	-0.31
	Lignin	0.15	0.56	0.26	-0.84***	0.49	-0.60*	-0.19	0.18	-0.17	-0.21	-0.26	-0.46**
	Root C:N ratio	0.26	0.37	0.09	-0.68*	0.29	-0.86***	-0.06	0.28	-0.07	-0.3	-0.17	-0.79***
	SIR	-0.16	-0.19	-0.11	0.65*	-0.57		0.27	-0.15	0.18	0.48**	0.12	

46

47 In bold, significant *r* values: * *p* < 0.05, ** *p* < 0.01, *** *p* < 0.001

48 **Fig. S1:** Spatial disposition of growth boxes.

49 In left panel, replicates are depicted in different colours according to their family (green: N₂-fixing
 50 Fabaceae, brown: non N₂-fixing Poaceae, yellow: non N₂ fixing *P. lanceolata*, blue: non N₂-fixing *S.*
 51 *minor*). The different shades of colour representing different species are shown in the legend. The blue
 52 dot on the upper right-hand corner of the different colored squares shows the boxes that were
 53 equipped with air/soil temperature and soil moisture sensors. Soil cores were removed from each row
 54 of 'soil core replicates' growth boxes. Half the boxes were fitted with rhizotrons ('rhizotron replicates').
 55 Photographs of each species can be seen in the bottom right panel of the figure.



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57

58 **Fig. S2:** Growth boxes used in the experiment.

59 Above a gravel layer, soil was homogeneously compacted into growth boxes. Seeds were sown at a
60 density of 155 plants m⁻². Panes of plexiglass on the front of the box allowed root elongation to be
61 observed over the 37 week long experiment.

62

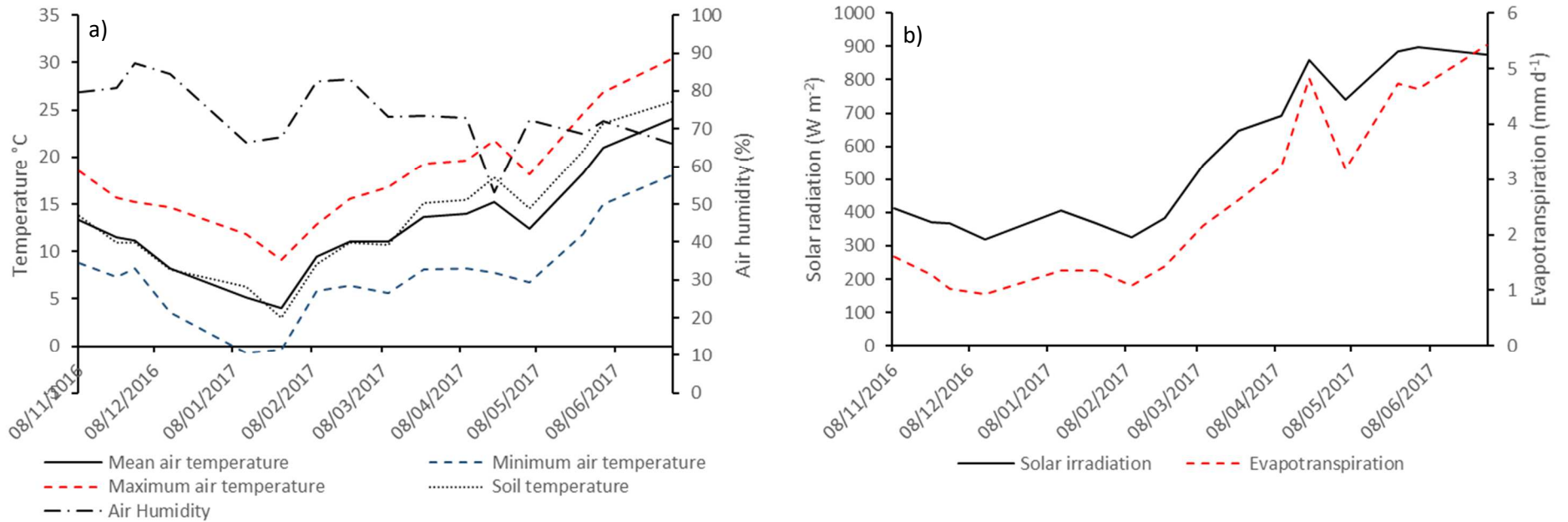


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65 **Fig. S3:** Climatic conditions over the 37 weeks of experimentation.

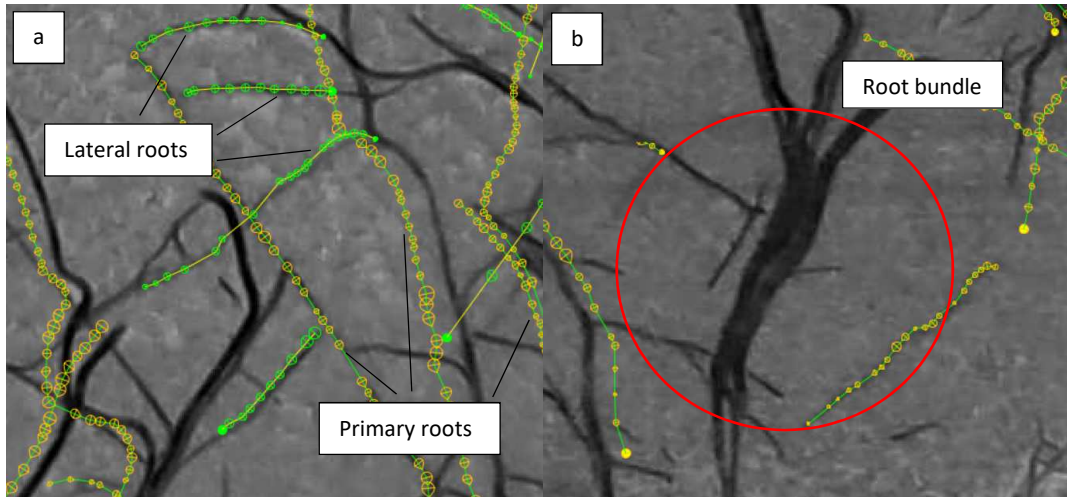
66 a) Mean daily air temperature (solid black line), minimum air temperature (segmented blue line), maximum air temperature (segmented red line), mean daily
67 soil temperature (dotted black line) and mean daily air humidity (segmented black, dotted line). In b), mean daily solar irradiation (solid black line) and
68 evapotranspiration (segmented red line).



69

70 **Fig. S4:** Example of an image analyzed using SmartRoot software.

71 a) primary roots (in orange) from which lateral roots are initiated (in green). Each orange circle along
72 the root axis represents a single 'mouse click' for root selection. b) A bundle of roots growing close
73 together, where the number and diameter of roots cannot be recognized by the SmartRoot software.

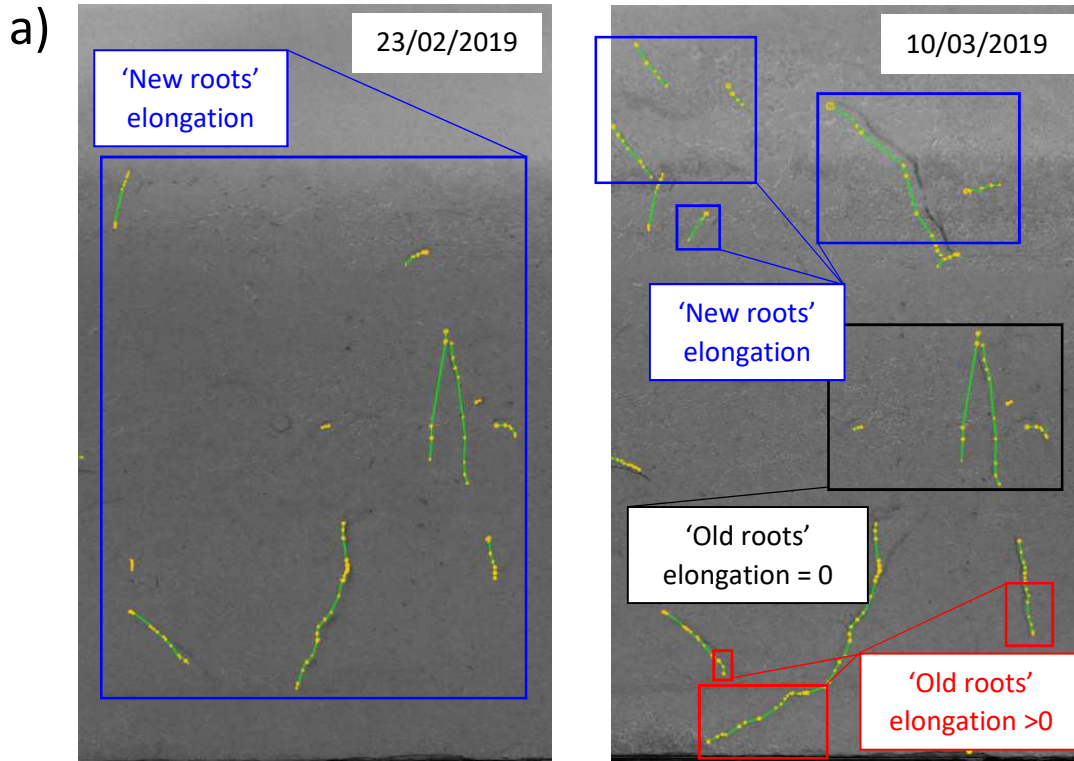


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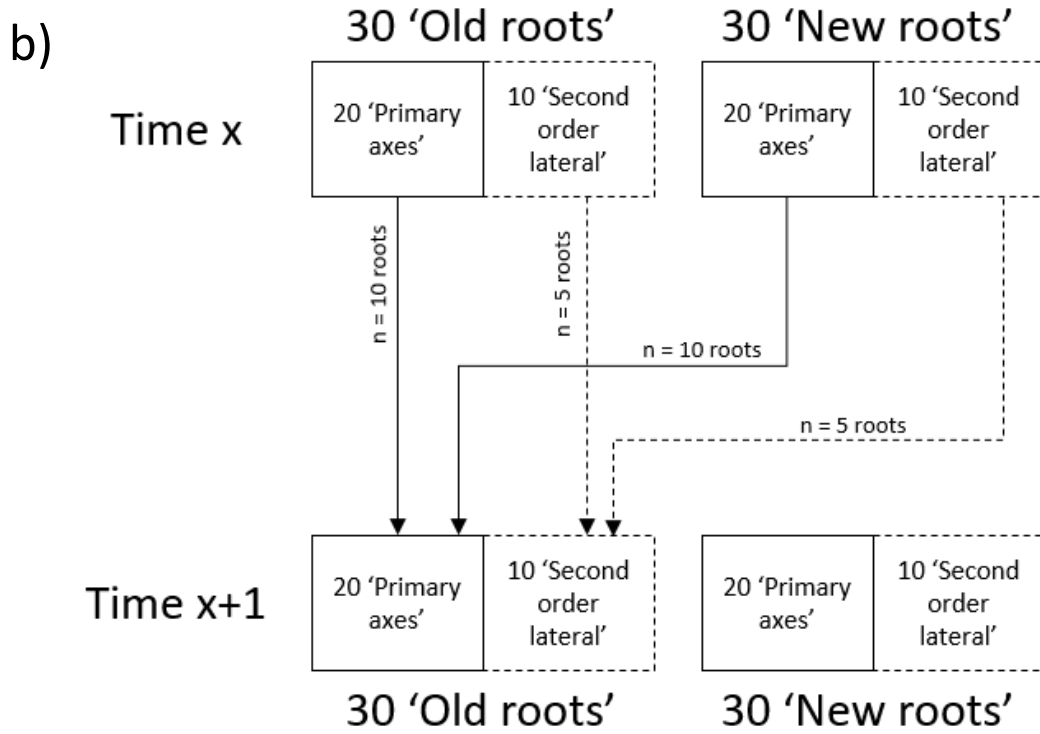
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76 **Fig.S5:** a) Example of two subsequent images of roots of *Bromus erectus* taken on 23/02/2019 and
77 10/03/2019 and b) conceptual scheme to explain root selection procedure..

78 a) The figure on the left shows newly initiated roots that will be analyzed to calculate the RER_{NEW} and
79 RLP_{NEW} on 23/02/2019. At the next date for image analysis (10/03/2019), some of the previously
80 analyzed roots were the same length ($RER=0$, middle of the rhizotron), whereas other roots elongated
81 ($RER>0$, bottom of the rhizotron), and were used to calculate RER_{OLD} and RLP_{OLD} . On the top part of the
82 rhizotron, some new roots were initiated, and analyzed to calculate the RER_{NEW} and RLP_{NEW} on
83 10/03/2019. b) Conceptual scheme showing the procedure to select 'old roots' at each sampling: at
84 Time x 30 new roots and 30 old roots have been analyzed. Of these 30 roots, 20 are primary axis roots
85 while 10 second order lateral (Fig. S4a). 10 roots from Time x primary old roots and 10 from Time x
86 primary new roots are randomly selected to be analyzed and constitute the Time x+1 old primary roots.
87 5 roots from Time x secondary old roots and 5 from Time x new secondary roots are selected and
88 analyzed and constitute the Time x+1 secondary old roots.



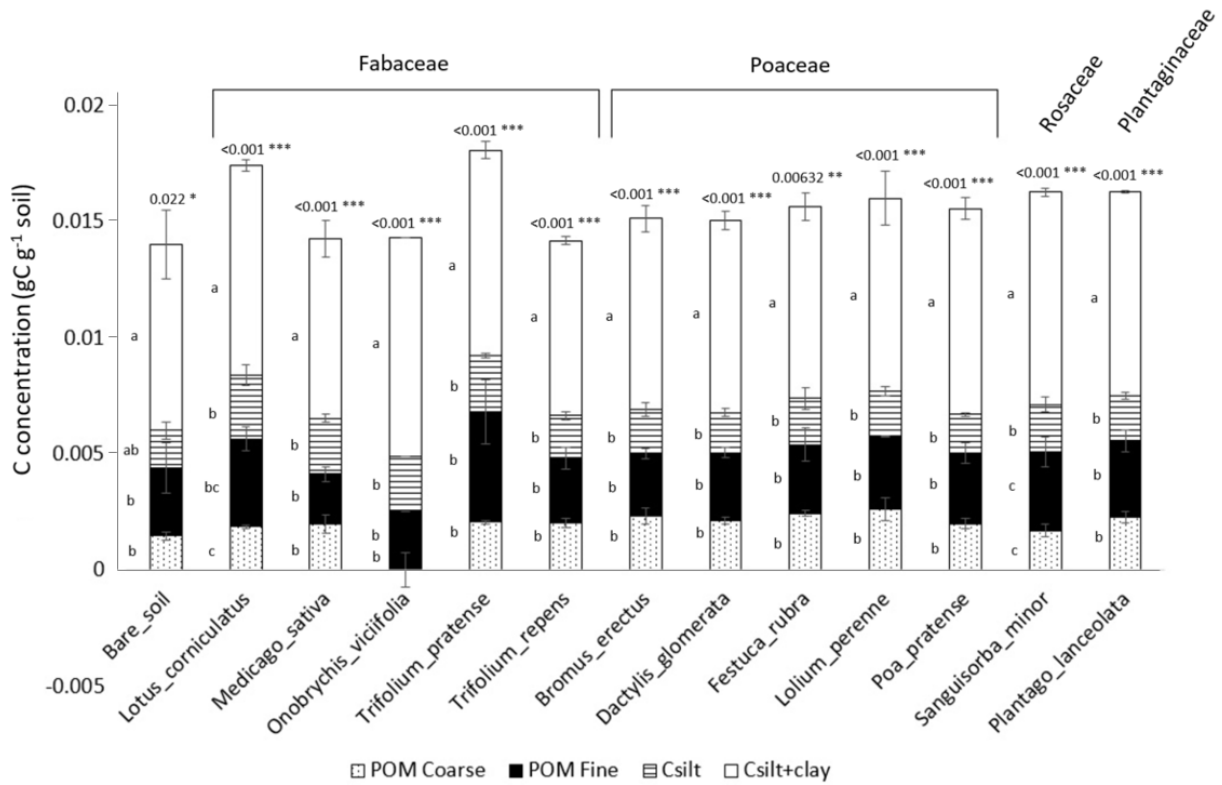
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91 **Fig. S6:** Carbon (C) content in each soil C pool beneath the 12 species and in the control bare soil, 37
 92 weeks after sowing.

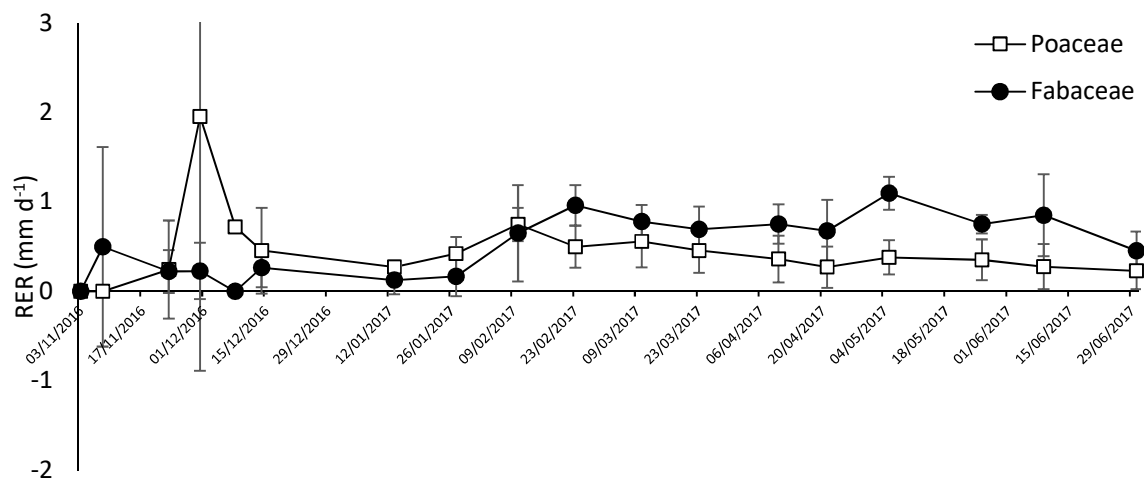
93 The C pools analyzed are C_{POM} in the coarse particulate organic matter $>200\mu\text{m}$), C_{finePOM} (C in the fine
 94 particulate organic matter $200\text{-}50\mu\text{m}$), C_{SILT} (in the coarse silt fraction $50\text{-}20\mu\text{m}$) and $C_{\text{SILT+CLAY}}$ (C in the
 95 fine silt + clay fraction $<20\mu\text{m}$). The letters on the left hand side of the fraction bars indicate significant
 96 differences (Tukey HSD, $p < 0.05$) between C pools and within species.



99 **Fig. S7:** Mean daily root elongation rate (RER_{TOT}) for all the roots analyzed in the rhizotrons (without
100 distinguishing between old and new roots) in Fabaceae (N_2 -fixing, black circles) and Poaceae (non N_2 -
101 fixing, white squares) family.

102 Mean daily RER_{TOT} in Fabaceae peaked in May - June, whereas in Poaceae, mean daily RER_{TOT} was fairly
103 constant between February and June, with no marked peaks. Data are means \pm standard error of the RER
104 data in the 2 weeks prior to the measurement of root elongation.

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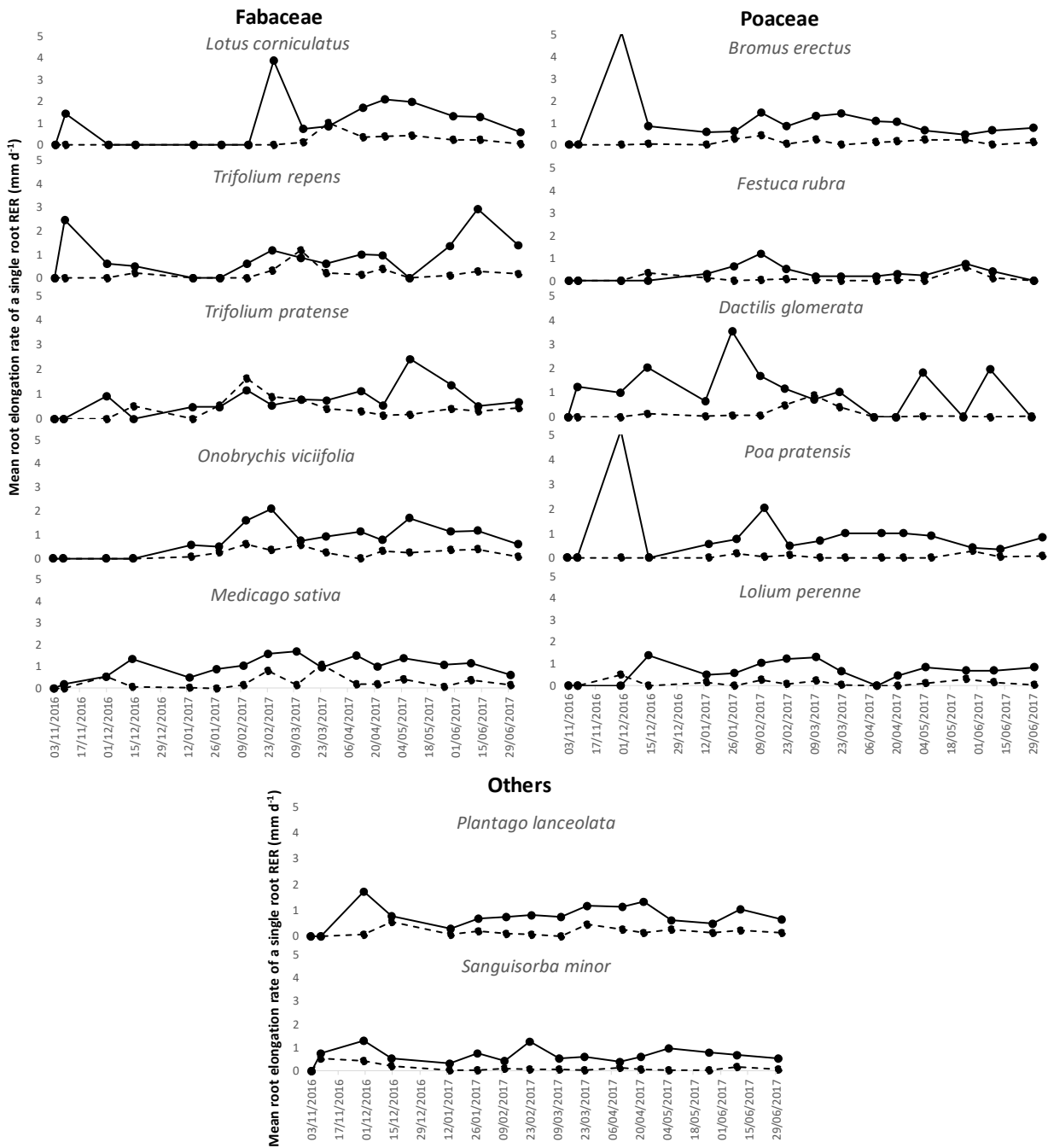


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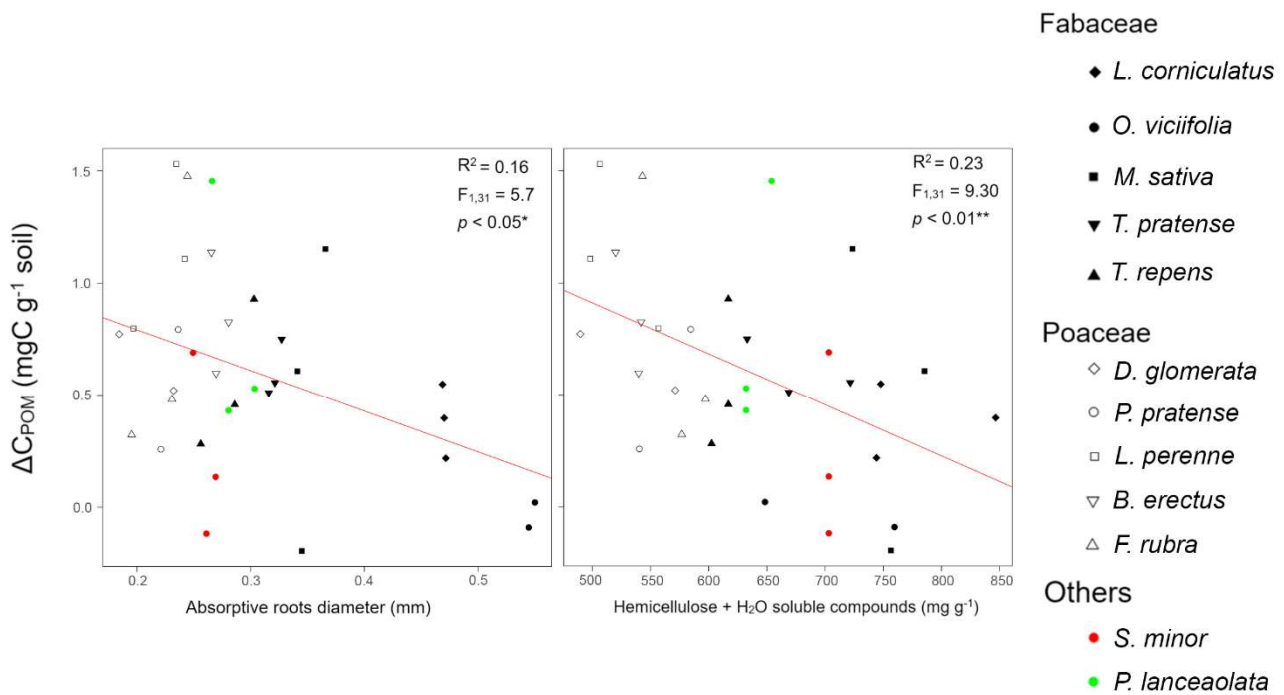
108 **Fig.S8:** Mean root elongation rate of individual roots (RER, measured every 2 weeks) for each of the 12
 109 species.

110 The solid black line is the RER_{NEW} of the roots that were newly initiated (aged 1 to 14 days), and were not
 111 present at the previous sampling date. The dotted black line represents the RER_{OLD} of the roots that were
 112 already present at the previous sampling date, and so were older than 14 days.



114 **Fig. S9:** Linear regression at the individual level (n = 34 samples), between ΔC_{POM} (as the difference in
 115 carbon (C) in the coarse POM C pool, $\Delta C = C_{t37} - C_{t0}$, after 37 weeks), and a) diameter of absorptive roots
 116 and b) hemicelluloses + water soluble compounds.

117 The black symbols are the N₂-fixing Fabaceae species, the white symbols the non N₂-fixing Poaceae
 118 species, the red dots are *S. minor* and the green dots are *P. lanceolata*. The red line is the linear model
 119 function of the variables and R², F and p of the linear model are shown.

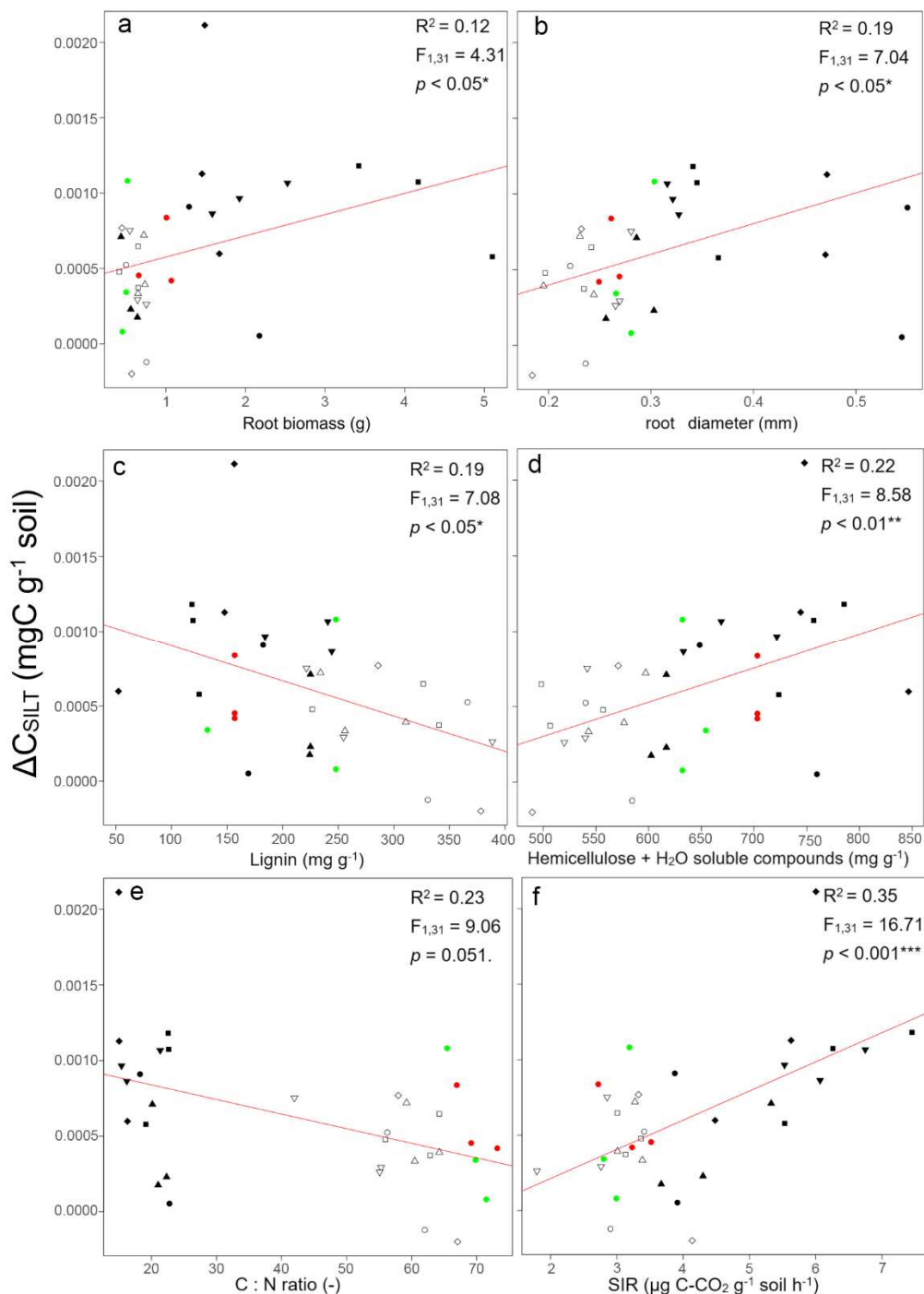


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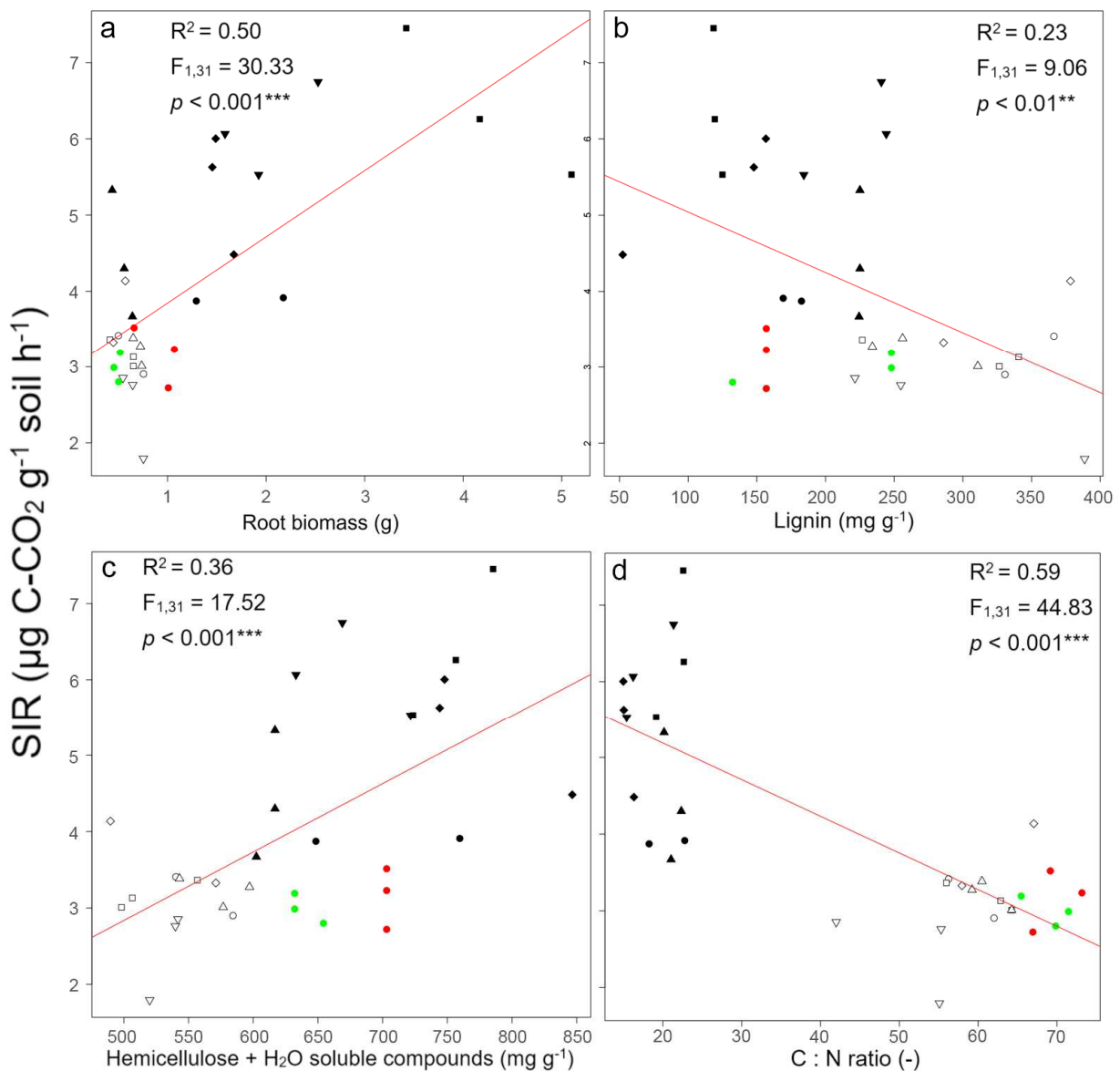
122 **Fig. S10:** Linear regression at individual level ($n = 34$ samples) between ΔC_{silt} (as the difference in carbon
 123 (C) in the coarse silt C pool, $\Delta C = C_{t37} - C_{t0}$, after 37 weeks), and a) root biomass, b) diameter of absorptive
 124 roots, c) lignin content, d) hemicelluloses + water soluble compounds, e) C:N ratio and f) substrate induced
 125 respiration rate (SIR).

126 The black symbols are the N_2 -fixing Fabaceae species, the white symbols the non N_2 -fixing Poaceae
 127 species, the red dots are *S. minor* and the green dots are *P. lanceolata*. The red line is the linear model
 128 function of the variables and R^2 , F and p of the linear model are shown. For the legend refer to figure S9.



129

130 **Fig. S11:** Linear regression at individual level (n = 34 samples) between substrate induced respiration rate
 131 (SIR) and a) root biomass, b) lignin content, c) hemicelluloses + water soluble compounds, d) C:N ratio.
 132 The black symbols are the N₂-fixing Fabaceae species, the white symbols the non N₂-fixing Poaceae
 133 species, the red dots *S. minor* and the green dots *P. lanceolata*. The red line is the linear model function
 134 of the variables and R², F and p of the linear model are shown. For the legend refer to figure S9.



135