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

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Supplementary Materials

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

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

Variables tested include: mean daily soil temperature, mean daily air temperature, minimum daily air temperature, maximum daily air temperature, mean 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 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 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**

		Soil temperature	Mean air temperature	Minimum air temperature	Maximum air teperature	Mean solar radiation
Poaceae	*Dactylis glomerata*	-0.23	-0.21	-0.1	-0.18	-0.1
	Lolium perenne	0.01	-0.04	-0.03	0.09	0.38
	Festuca rubra	-0.21	-0.27	-0.34	-0.2	0.15
	Bromus erectus	-0.02	-0.05	-0.01	0.02	0.29
	Poa pratensis	0.1	0.08	0.34	0.1	0.43
Fabaceae	*Trifolium repens*	**0.76*** **	**0.81*** **	0.45	**0.68****	0.33
	Trifolium pratense	0.02	-0.02	-0.02	0.09	0.4
	Lotus corniculatus	**0.70****	**0.70****	0.44	**0.68****	**0.72****
	Onobrychis viciifolia	0.13	0.09	0.3	0.17	**0.54***
	Medicago sativa	0.01	0.01	-0.05	0.02	0.22
Rosaceae	*Sanguisorba minor*	-0.06	-0.06	-0.06	-0.04	0.16
Plantaginaceae	*Plantago lanceolata*	0	-0.04	0	0.04	0.3

b - Correlation between climate factors and RER_{OLD} of the **old roots**

Family	Species	Soil temperature	Mean air temperature	Minimum air temperature	Maximum air teperature	Mean solar radiation
Poaceae	*Dactylis glomerata*	-0.27	-0.26	-0.17	-0.22	-0.12
	Lolium perenne	0.14	0.13	0.31	0.09	0.21
	Festuca rubra	-0.25	-0.3	-0.41	-0.25	0.05
	Bromus erectus	0.17	0.28	-0.07	0.2	0.29
	Poa pratensis	-0.03	0	0.4	0.04	0.21
Fabaceae	*Trifolium repens*	0.28	0.28	0.15	0.4	0.45
	Trifolium pratense	0.18	0.25	-0.07	0.24	0.38
	Lotus corniculatus	**0.73****	**0.71****	0.51	**0.74****	**0.89*** **
	Onobrychis viciifolia	0.04	0.02	0.26	0.07	0.36
	Medicago sativa	0.03	0.01	-0.05	0.03	0.23
Rosaceae	*Sanguisorba minor*	0.13	0.2	-0.08	0.1	-0.33
Plantaginaceae	*Plantago lanceolata*	-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 teperature	Mean solar radiation
Poaceae	*Dactylis glomerata*	**-0.55***	**-0.54***	-0.47	**-0.60***	**-0.66****
	Lolium perenne	0.03	0	-0.05	0.1	0.29
	Festuca rubra	-0.11	-0.16	-0.04	-0.15	0.26
	Bromus erectus	-0.06	-0.07	-0.08	-0.05	0.17
	Poa pratensis	0.09	0.08	0.36	0.06	0.38
Fabaceae	*Trifolium repens*	**0.59***	**0.67****	0.17	**0.57***	0.17
	Trifolium pratense	0.11	0.1	0.37	0.08	0.41
	Lotus corniculatus	**0.76*** **	**0.75****	0.48	**0.76*** **	**0.76****
	Onobrychis viciifolia	0.13	0.1	0.3	0.15	0.51
	Medicago sativa	0.04	0.06	-0.35	0.04	0.21
Rosaceae	*Sanguisorba minor*	0.09	0.16	0.04	0.06	-0.05
Plantaginaceae	*Plantago lanceolata*	-0.05	-0.06	-0.06	-0.03	0.19

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

Variables tested include: mean daily soil temperature, mean daily air temperature, minimum daily air temperature, maximum daily air temperature, mean 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 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 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 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 teperature	Average solar radiation
Poaceae	*Dactylis glomerata*	-0.4	-0.41	-0.46	-0.45	-0.38
	Lolium perenne	-0.02	-0.05	-0.08	0.04	0.29
	Festuca rubra	-0.19	-0.25	-0.32	-0.18	0.18
	Bromus erectus	-0.01	-0.05	0.01	0.03	0.31
	Poa pratensis	0.12	0.09	0.35	0.12	0.46
Fabaceae	*Trifolium repens*	0.36	0.37	-0.01	0.33	0.38
	Trifolium pratense	0.03	0	0.03	0.07	0.34
	Lotus corniculatus	**0.72****	**0.72****	0.46	**0.70****	**0.74****
	Onobrychis viciifolia	0.13	0.09	0.3	0.17	**0.54***
	Medicago sativa	0.01	-0.01	-0.04	0.02	0.24
Rosaceae	*Sanguisorba minor*	-0.06	-0.07	-0.05	-0.04	0.18
Plantaginaceae	*Plantago lanceolata*	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 teperature	Mean solar radiation
Poaceae	*Dactylis glomerata*	-0.29	-0.34	-0.38	-0.28	0
	Lolium perenne	0	-0.07	-0.02	-0.08	0.24
	Festuca rubra	-0.16	-0.22	-0.31	-0.15	0.19
	Bromus erectus	0.18	0.25	0	0.23	0.38
	Poa pratensis	0.02	0.03	0.4	0.1	0.31
Fabaceae	*Trifolium repens*	0.28	0.26	0.2	0.41	**0.51***
	Trifolium pratense	0.22	0.26	0.02	0.29	0.47
	Lotus corniculatus	**0.72****	**0.69****	0.52	**0.75*****	**0.90*****
	Onobrychis viciifolia	0.06	0.03	0.28	0.1	0.42
	Medicago sativa	0.04	0	0.02	0.08	0.33
Rosaceae	*Sanguisorba minor*	-0.04	-0.05	-0.06	-0.02	0.18
Plantaginaceae	*Plantago lanceolata*	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 teperature	Mean solar radiation
Poaceae	*Dactylis glomerata*	**-0.52***	**-0.51***	-0.51	**-0.54***	-0.48
	Lolium perenne	-0.25	-0.3	-0.43	-0.27	0.04
	Festuca rubra	-0.08	-0.14	-0.03	-0.1	0.33
	Bromus erectus	0.01	-0.03	0.01	0.05	0.33
	Poa pratensis	0.13	0.1	0.34	0.12	0.46
Fabaceae	*Trifolium repens*	**0.51***	**0.57***	0.11	0.49	0.26
	Trifolium pratense	0.17	0.14	0.36	0.17	**0.51***
	Lotus corniculatus	**0.69****	**0.69****	0.41	**0.70****	**0.77*****
	Onobrychis viciifolia	0.06	0.01	0.02	0.13	0.43
	Medicago sativa	0.03	0.02	-0.03	0.05	0.27
Rosaceae	*Sanguisorba minor*	0.17	0.14	0.19	0.22	0.46
Plantaginaceae	*Plantago lanceolata*	0.01	-0.03	0.01	0.05	0.32

Table S3: Pearson's correlation coefficients (*r*) showing relationships between root variables and soil variables. Table S3a) shows the significant correlations 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 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 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 biomass of a core sampled at 37 weeks; absorptive root diameter– mean diameter of absorptive roots at 37 weeks; hemicellulose + water soluble compounds – concentration of hemicellulose and water soluble compounds in absorptive roots; cellulose, lignin – concentrations of cellulose and lignin in absorptive 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 $+H_2O$ 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	

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

Fig. S1: Spatial disposition of growth boxes.

In left panel, replicates are depicted in different colours according to their family (green: N_2-fixing Fabaceae, brown: non N_2-fixing Poaceae, yellow: non N_2 fixing *P. lanceolata*, blue: non N_2-fixing *S. minor*). The different shades of colour representing different species are shown in the legend. The blue dot on the upper right-hand corner of the different colored squares shows the boxes that were equipped with air/soil temperature and soil moisture sensors. Soil cores were removed from each row of 'soil core replicates' growth boxes. Half the boxes were fitted with rhizotrons ('rhizotron replicates'). Photographs of each species can be seen in the bottom right panel of the figure.

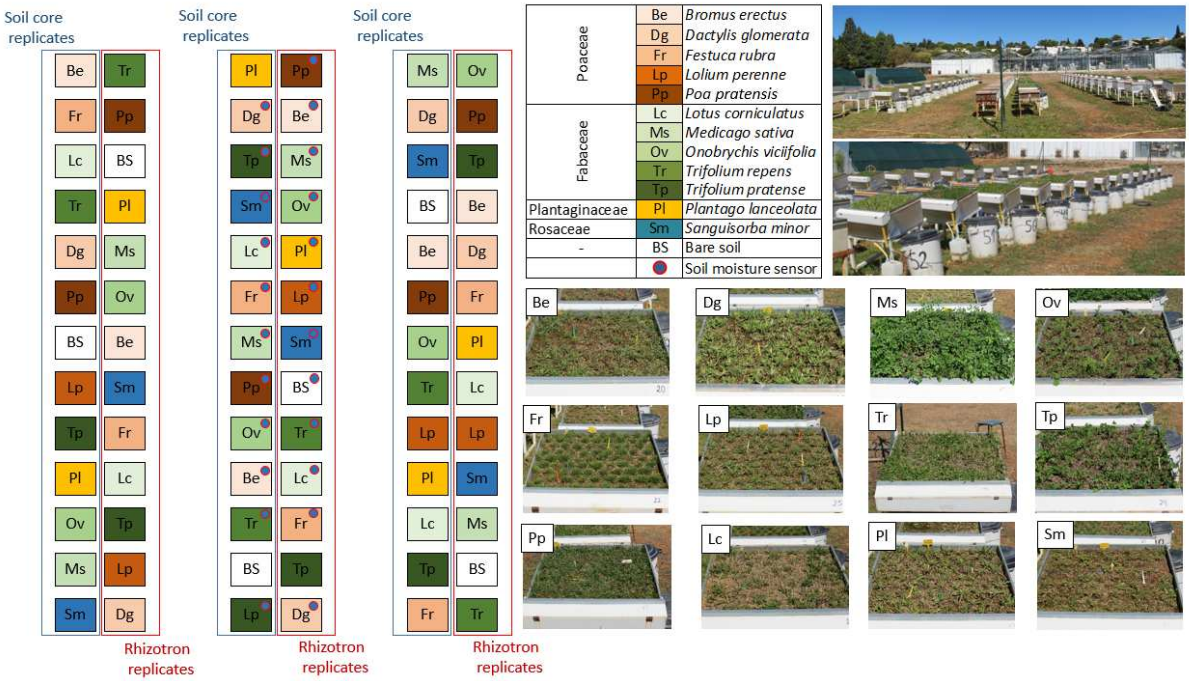


Fig. S2: Growth boxes used in the experiment.

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

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

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

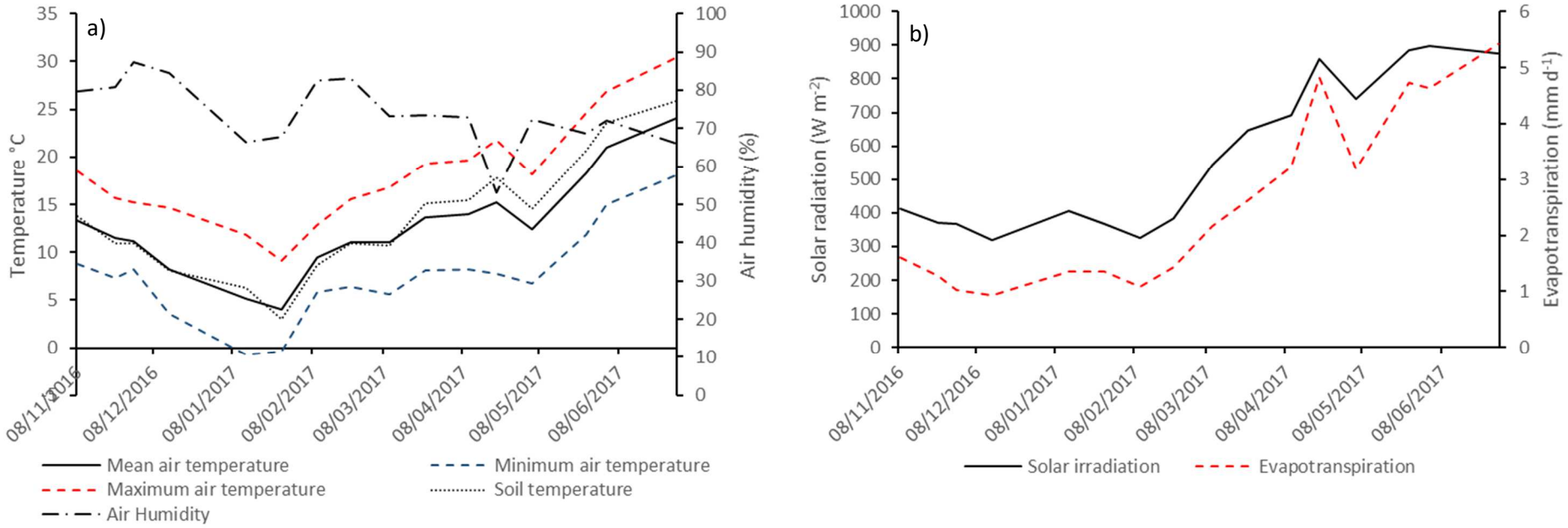


Fig. S4: Example of an image analyzed using SmartRoot software.

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

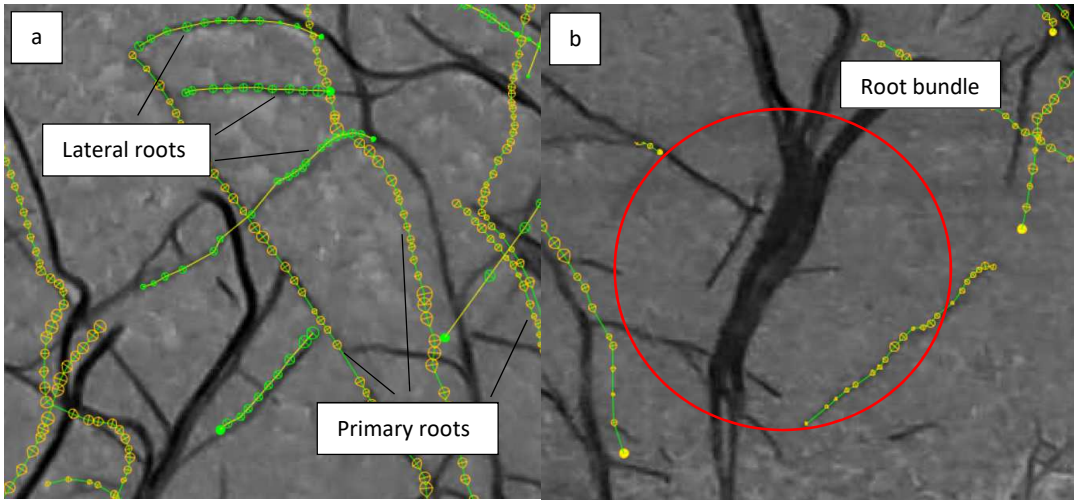
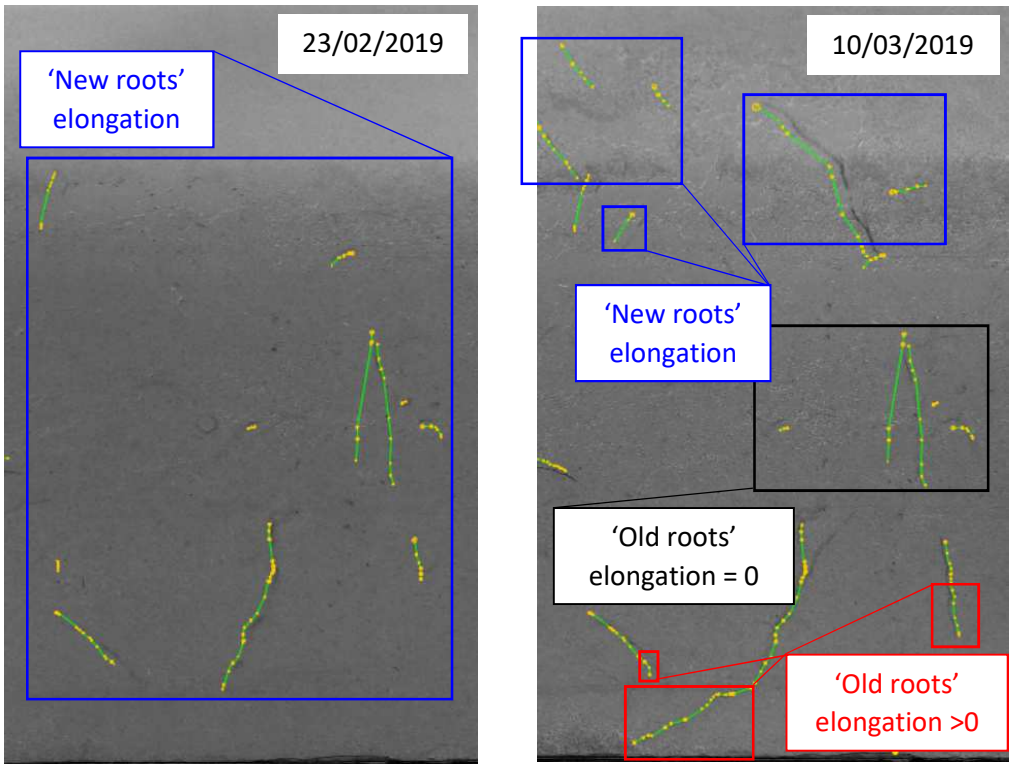


Fig.S5: a) Example of two subsequent images of roots of *Bromus erectus* taken on 23/02/2019 and 10/03/2019 and b) conceptual scheme to explain root selection procedure..

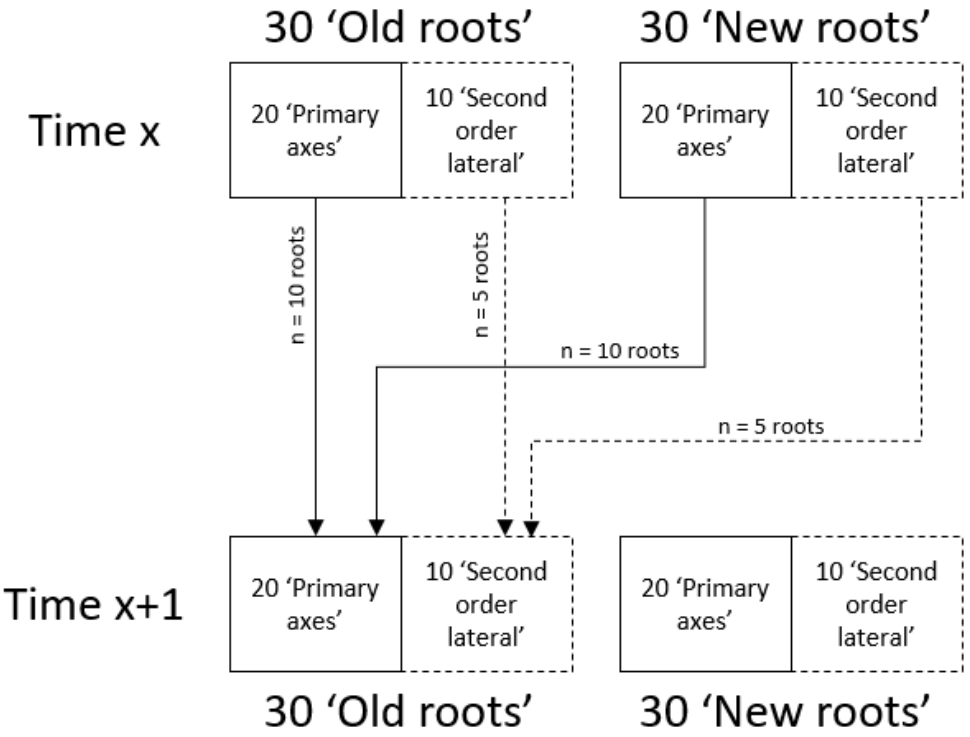
a) The figure on the left shows newly initiated roots that will be analyzed to calculate the RER_{NEW} and RLP_{NEW} on 23/02/2019. At the next date for image analysis (10/03/2019), some of the previously analyzed roots were the same length (RER=0, middle of the rhizotron), whereas other roots elongated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

a)


23/02/2019
'New roots' elongation
10/03/2019
'New roots' elongation
'Old roots' elongation = 0
'Old roots' elongation >0

89

b)


30 'Old roots'
30 'New roots'
Time x
20 'Primary axes'
10 'Second order lateral'
20 'Primary axes'
10 'Second order lateral'
n = 10 roots
n = 5 roots
n = 10 roots
n = 5 roots
Time x+1
20 'Primary axes'
10 'Second order lateral'
20 'Primary axes'
10 'Second order lateral'
30 'Old roots'
30 'New roots'

90

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

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

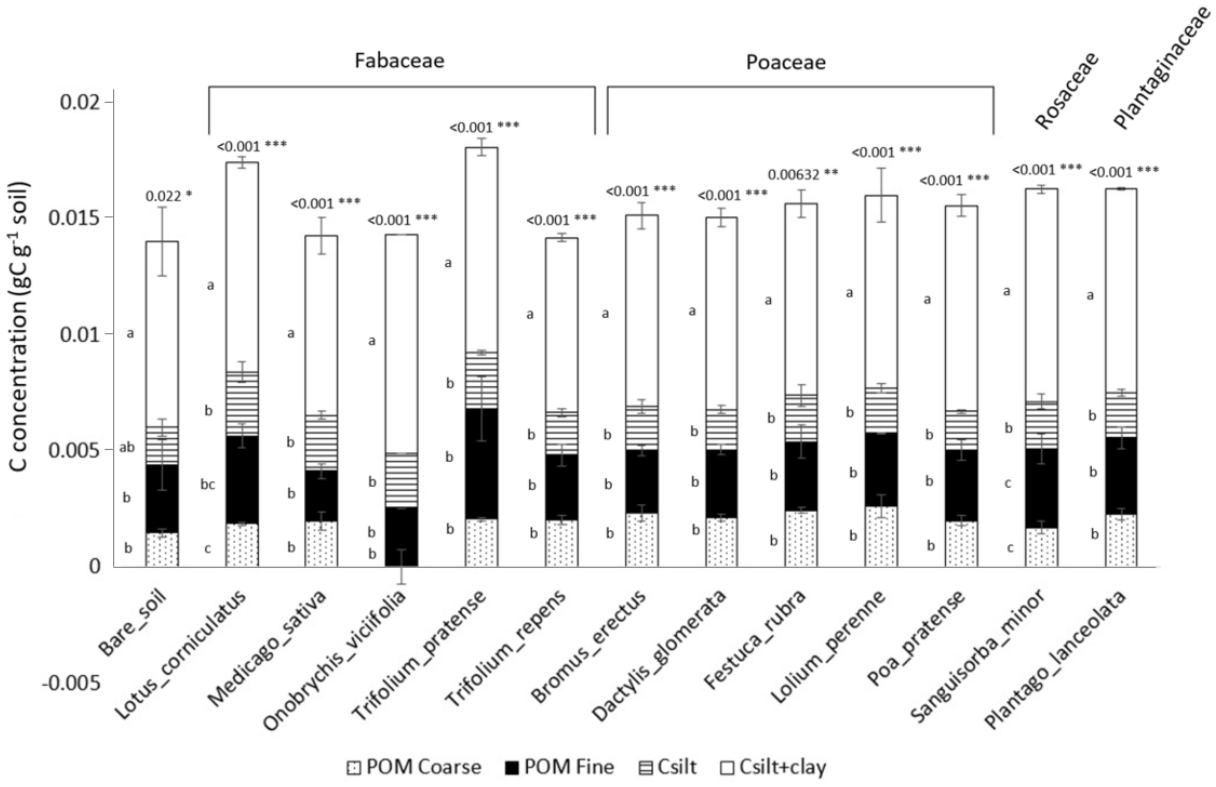


Fig. S7: Mean daily root elongation rate (RER_{TOT}) for all the roots analyzed in the rhizotrons (without distinguishing between old and new roots) in Fabaceae (N_2-fixing, black circles) and Poaceae (non N_2-fixing, white squares) family.

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

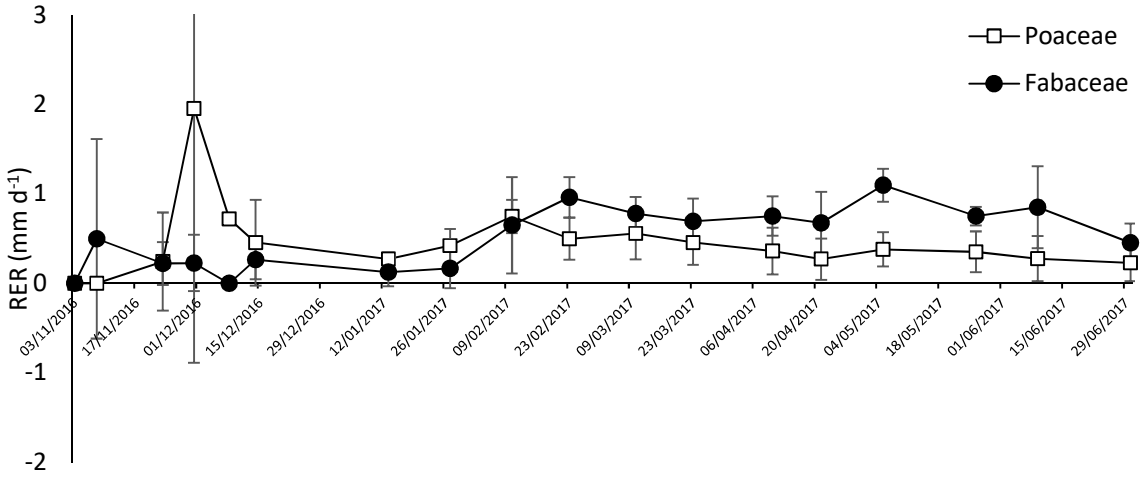


Fig.S8: Mean root elongation rate of individual roots (RER, measured every 2 weeks) for each of the 12 species.

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

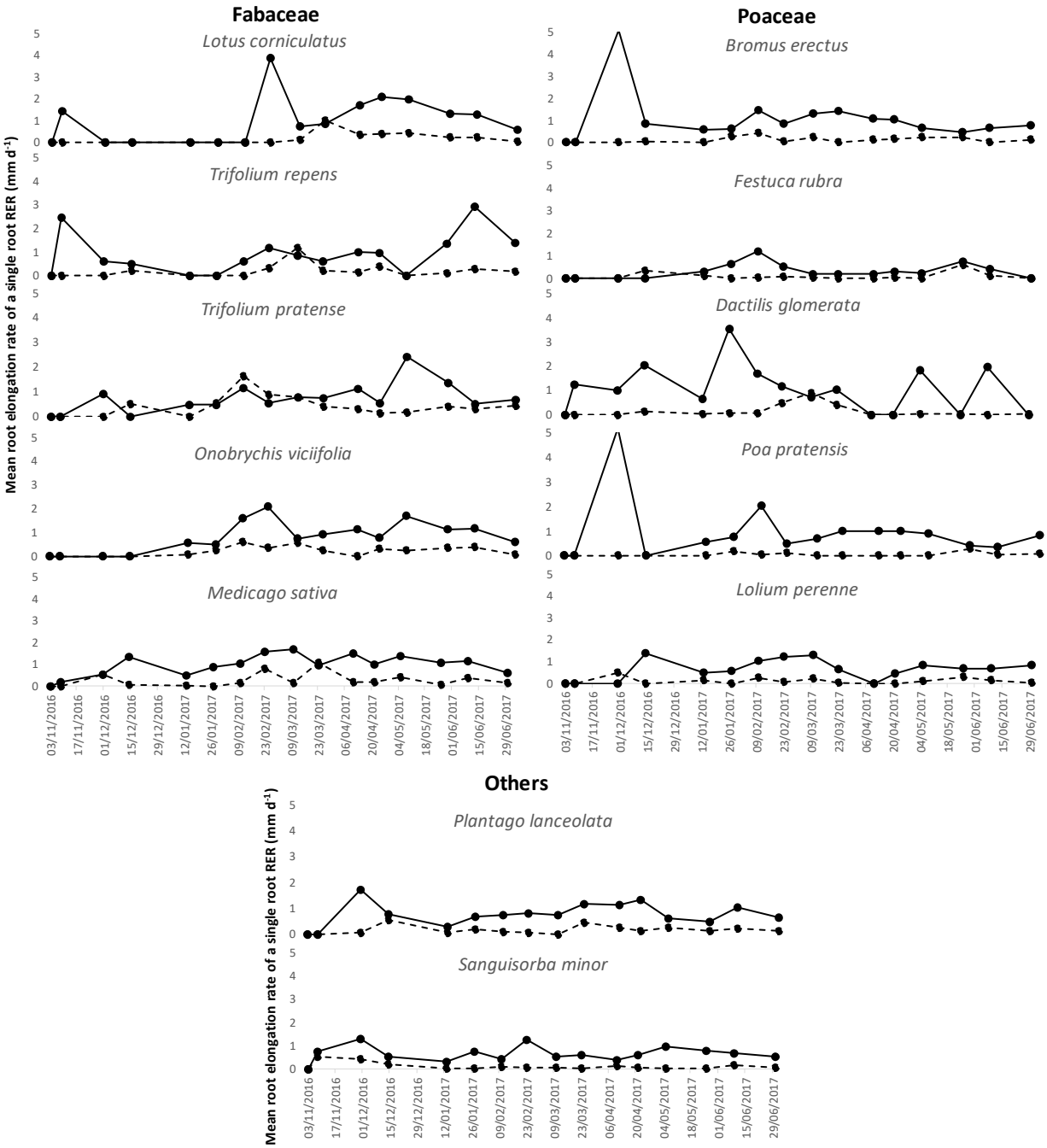


Fig. S9: Linear regression at the individual level (n = 34 samples), between ΔC_{POM} (as the difference in carbon (C) in the coarse POM C pool, $\Delta C = C_{t37} - C_{t0}$, after 37 weeks), and a) diameter of absorptive roots and b) hemicelluloses + water soluble compounds.

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.

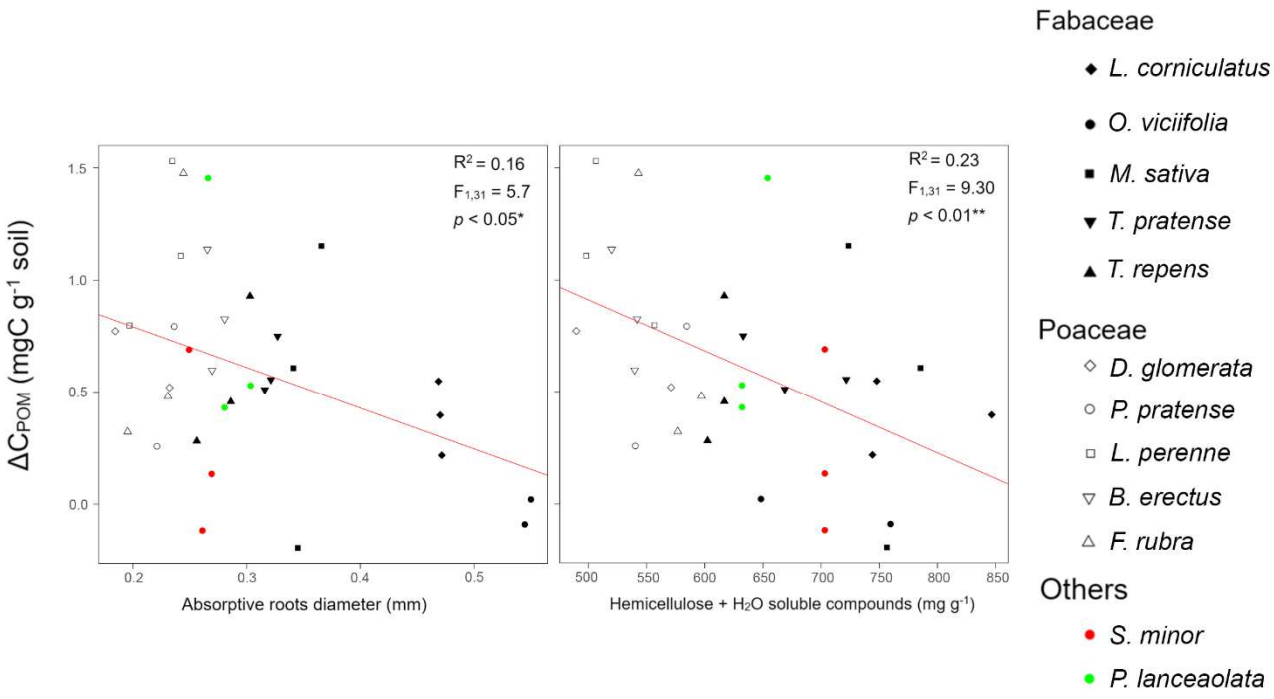


Fig. S10: Linear regression at individual level (n = 34 samples) between ΔC_{silt} (as the difference in carbon (C) in the coarse silt C pool, $\Delta C = C_{t37} - C_{t0}$, after 37 weeks), and a) root biomass, b) diameter of absorptive roots, c) lignin content, d) hemicelluloses + water soluble compounds, e) C:N ratio and f) 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. For the legend refer to figure S9.

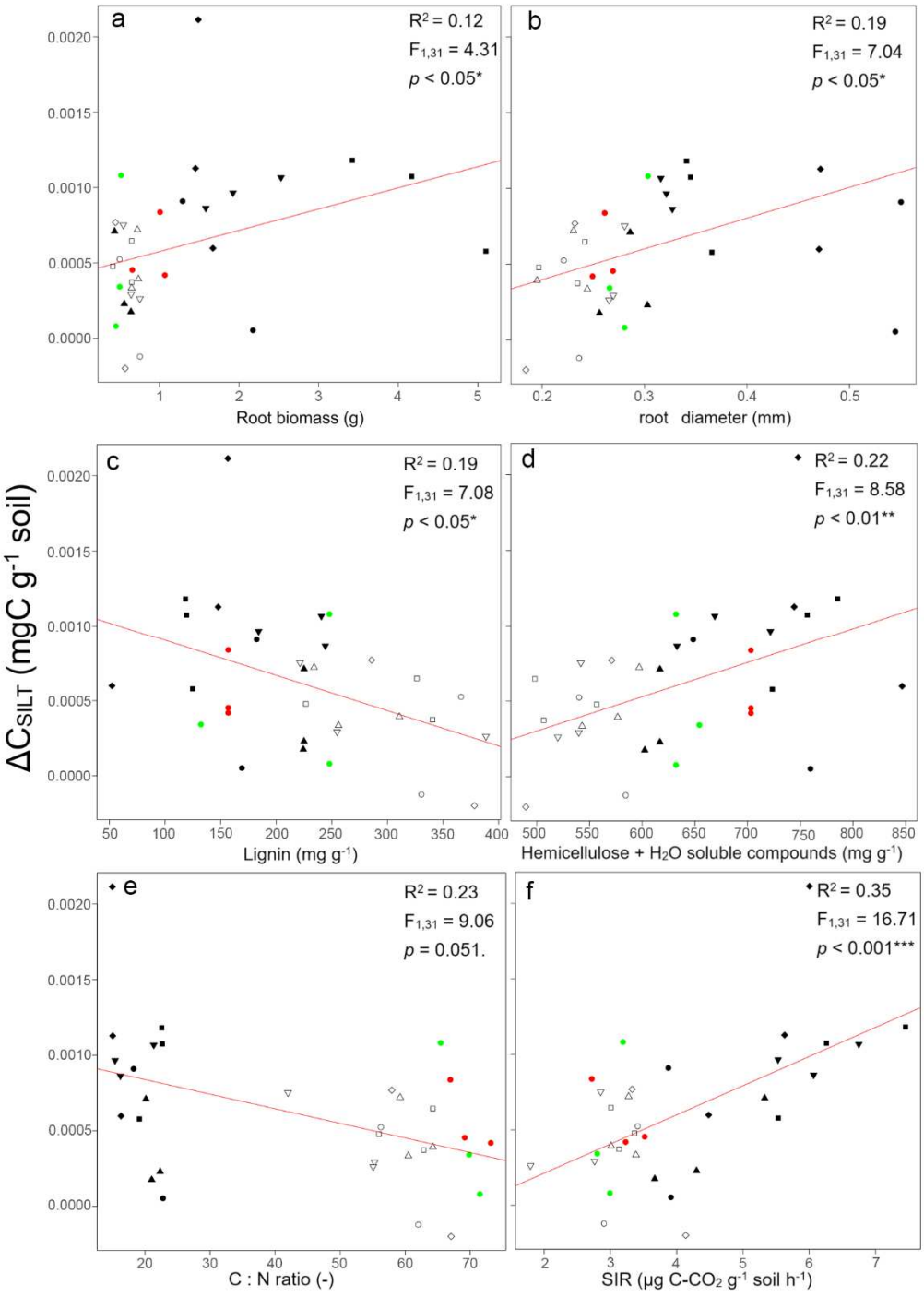


Fig. S11: Linear regression at individual level (n = 34 samples) between substrate induced respiration rate (SIR) and a) root biomass, b) lignin content, c) hemicelluloses + water soluble compounds, d) C:N ratio.

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. For the legend refer to figure S9.