



Supplement of

Plant clustering generates negative plant–soil feedback without changing the spatial distribution of soil fauna

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1 Supplementary material:

2 **Text S1: Material and methods**

3 *Mesocosm preparation and soil characteristics*

4 The containers were surface sterilized by washing with 10% household bleach and then rinsed
5 three times with ample tap water. This procedure was repeated 5 times. After that, the surface
6 was cleaned with 70% ethanol, which was left to evaporate for 20 minutes before the next
7 surface cleaning. This procedure was also repeated 5 times, after which sterilised soil mixture
8 was added. Approximately 42.5 kg of soil mixture was added to each container with an
9 average water content of 27%. We used a mixture of garden soil (Structural, Belgium) and
10 sand (Decor Son, Netherlands) with a volume ratio of 2:1 (garden soil: sand). This soil mixture
11 was sterilized by autoclaving at 121 °C for 30 minutes. The garden soil had 25% organic matter
12 and a pH of 5-6.5. Electrical conductivity was 300 µs/cm. The garden soil also contained 1.25
13 kg/m³ composite NPK (14-16-18) fertilizer.

14

15 *Watering regime of the mesocosms:*

16 The 10 mesocosms were randomly organised in a plant-growing room with a light/dark
17 regime of 16 h/8 h and 22 °C constant room temperature. The watering method changed
18 from spraying to direct addition from 45 days (i.e., third sampling timepoint) onwards after
19 the start of the experiment. Specifically, 1350 ml of distilled water was carefully and evenly
20 sprayed over the soil surface of each mesocosm (60*80 cm²) every two days until day 45.
21 Gentle spraying ensured the added water did not cause any passive dispersal of soil
22 nematodes and rotifers. 2700 ml of distilled water was evenly added to the mesocosms every
23 two days by adding 675 ml into the mesocosms in 4 batches, each from a different direction,
24 i.e., a different side of the mesocosm from day 45 to day 75. 2000 ml of distilled water was
25 evenly added the same way to the mesocosms every two days from the day 75 to day 90.
26 This higher frequency and larger amount of water per plant for the watering at later times

27 was to ensure no drought stress occurred as the plants grew larger. Moisture was checked
28 one day after each water addition by using garden combi testers (TFA Dostmann, Germany)
29 and soil water content was checked after each plant sampling by drying 20 grams of soil at
30 100 °C for 48 hours. After that, water was added to the mesocosms based on water content
31 and known dry soil weight to set the water content to the average of all 10 mesocosms at a
32 target level of 25% water content.

33

34 Tables and figures

35 Table S1: Coordinates for the nine plant nodes (L1-L9) of each of the 10 mesocosms with spatial
 36 positions of nodes ranging from clustered over random to regular.

R	Configuration	L1	L2	L3	L4	L5	L6	L7	L8	L9
0.6	Clustered	[40, 30]	[65, 31]	[51, 54]	[36, 26]	[42, 52]	[60, 20]	[67, 31]	[48, 49]	[26, 47]
0.72		[40, 30]	[50, 22]	[58, 33]	[46, 53]	[48, 20]	[14, 12]	[56, 42]	[46, 28]	[21, 21]
0.77		[40, 30]	[56, 41]	[54, 48]	[29, 43]	[45, 15]	[49, 39]	[54, 42]	[21, 37]	[8, 44]
0.86		[40, 30]	[15, 28]	[25, 25]	[30, 32]	[18, 42]	[66, 32]	[50, 18]	[12, 25]	[41, 17]
0.92		[40, 30]	[22, 31]	[59, 17]	[63, 38]	[51, 21]	[47, 56]	[29, 24]	[23, 37]	[47, 30]
1.02	Random	[40, 30]	[61, 55]	[32, 30]	[17, 28]	[53, 24]	[60, 28]	[21, 52]	[44, 34]	[41, 38]
1.09		[40, 30]	[59, 19]	[29, 17]	[25, 37]	[57, 42]	[21, 15]	[20, 29]	[39, 15]	[41, 46]
1.13		[40, 30]	[55, 56]	[31, 38]	[23, 20]	[49, 35]	[43, 20]	[63, 23]	[51, 25]	[23, 47]
1.18		[40, 30]	[76, 40]	[17, 35]	[38, 19]	[27, 26]	[47, 49]	[50, 18]	[33, 36]	[50, 29]
1.29		Regular	[40, 30]	[20, 15]	[20, 30]	[20, 45]	[40, 15]	[40, 45]	[60, 15]	[60, 30]

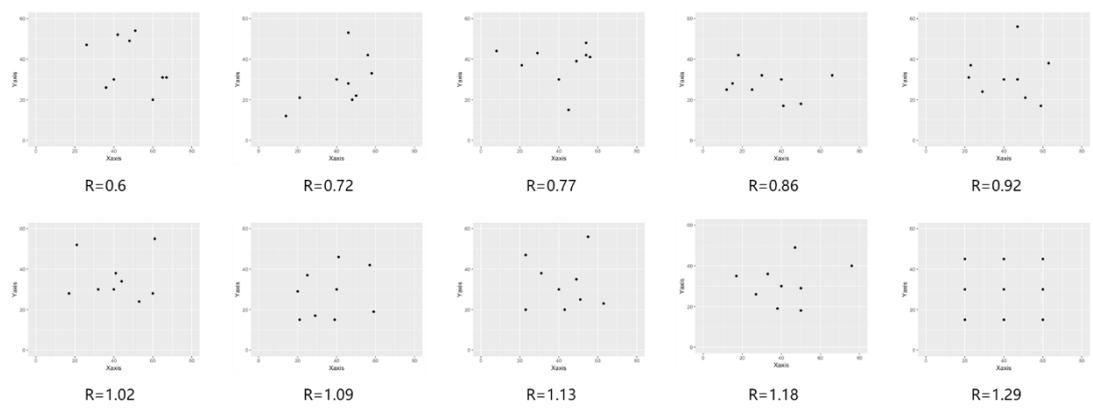
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38 Table S2: Abundance of each soil fauna functional group from five subsamples of 2.5 ml of the
 39 inoculum extracted from field *A. stolonifera* roots and rhizosphere soil and calculated abundance of
 40 each functional group extrapolated to the inoculum used per plant and per plant node (6 plants).

	BF	FF	PF	PRE	ROT	OMN	ENC	UK	Volume
Sample1	167	18	31	34	36	3	4	9	2.5ml
Sample2	163	15	37	51	50	3	2	8	2.5ml
Sample3	144	10	21	37	84	3	4	8	2.5ml
Sample4	116	13	27	34	100	0	2	6	2.5ml
Sample5	147	11	36	41	30	4	4	8	2.5ml
Mean	147.4	13.4	31	40	60	3	3	8	2.5ml
SD	20.16	3.21	6.62	7.09	30.63	1.52	1.10	1.10	2.5ml
SE	9.01	1.44	2.96	3.17	13.70	0.68	0.49	0.49	2.5ml
Per Plant	117.92	10.72	24.32	31.52	48	2.08	2.56	6.24	2ml
Per Node	707.52	64.32	145.92	189.12	288	12.48	15.36	37.44	12ml

41 BF: bacteria-feeding nematodes; FF: fungi-feeding nematodes; PF: plant-feeding nematodes; PRE:
 42 predatory nematodes; ROT: rotifers; OMN: omnivorous nematodes; ENC: enchytraeids; UK: unknown;
 43 SD: standard deviation; SE: standard error.

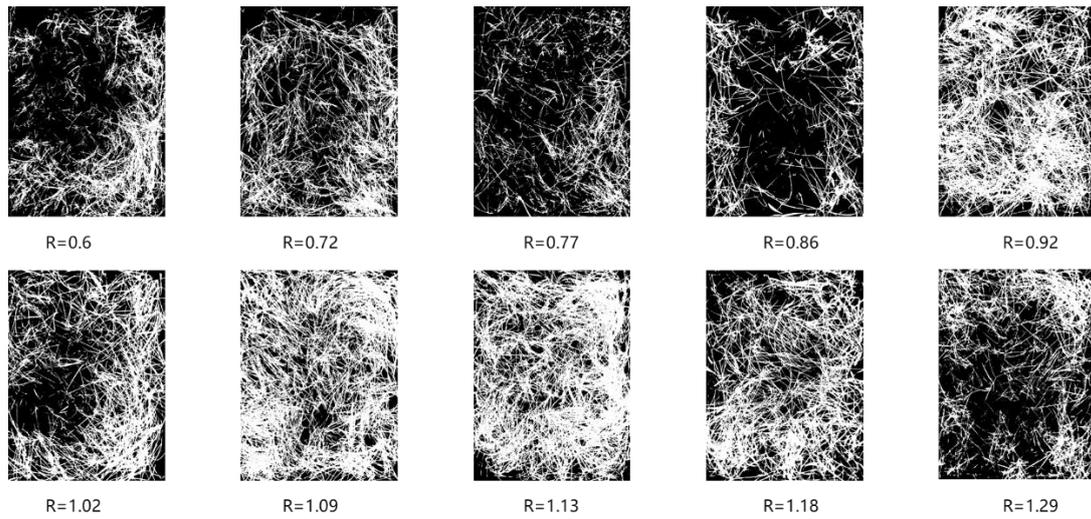
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46 Figure S1: The positions of the nine nodes in each mesocosm with a dimension of 60 by 80 cm. The R-
47 value indicates the regularity of the spatial network, with lower values corresponding with more
48 clustered, and higher values with more regular configurations

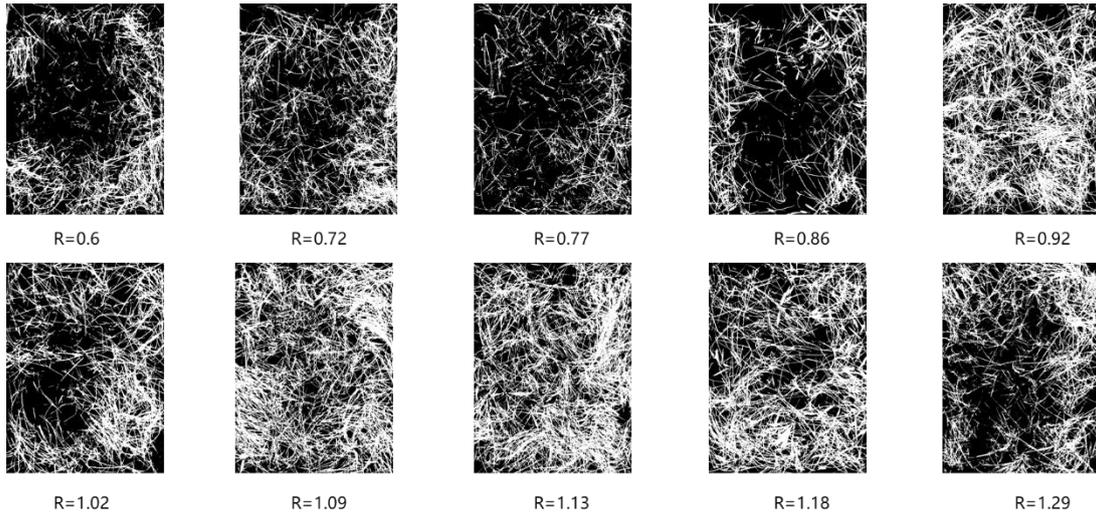
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51 Figure S2: Photos of cover by the subsequent vegetation of *L. perenne* and *P. lanceolata*
52 taken at 80 cm above each mesocosm at the 4th measurement time (6 weeks after sowing the seeds). The R-
53 value indicates the regularity of the spatial network, with lower values corresponding with more
54 clustered, and higher values with more regular configurations

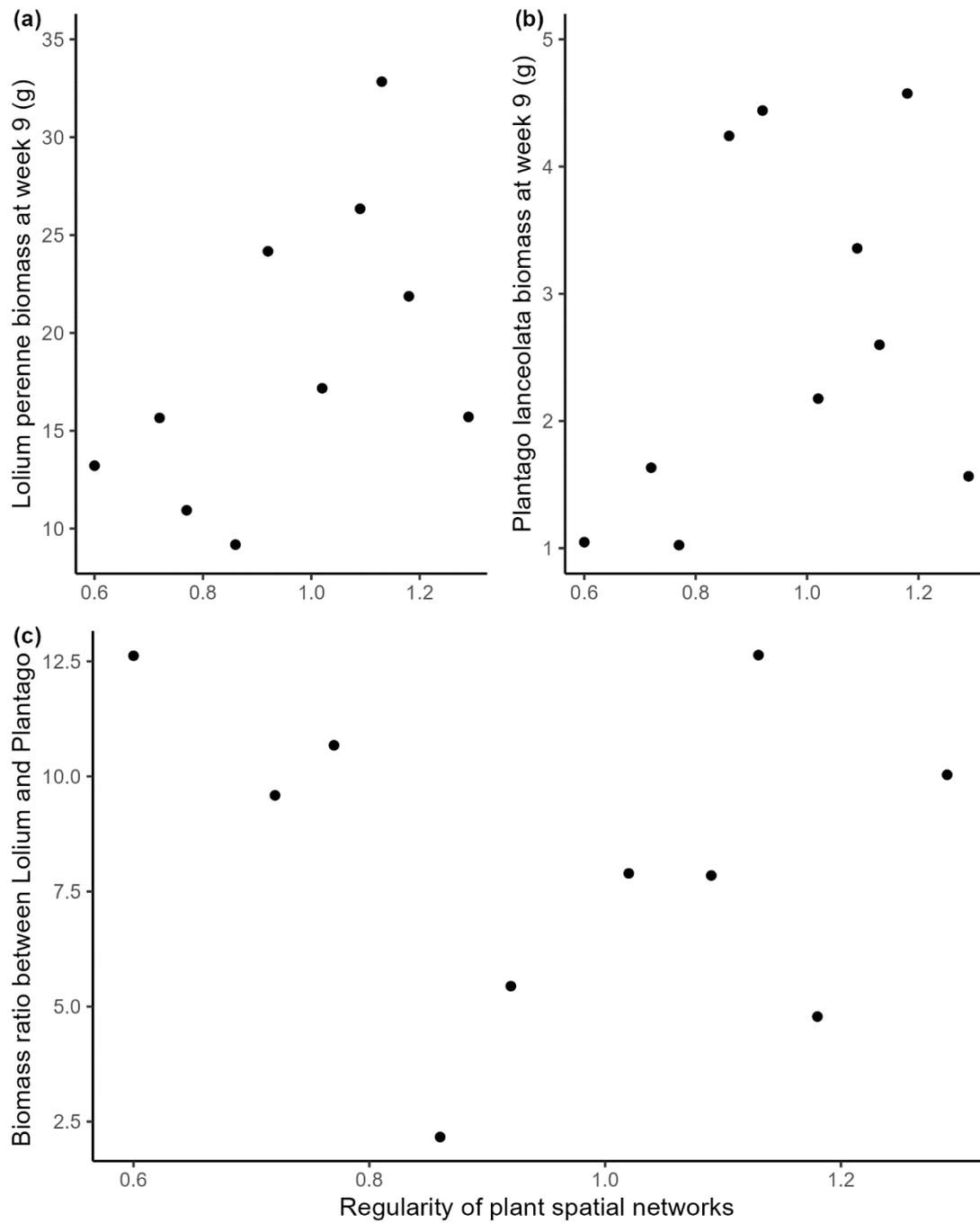
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56

57 Figure S3: Photos of cover by the subsequent vegetation of *L. perenne* and *P. lanceolata* taken at 80
 58 cm above each mesocosm at the 7th measurement time (9 weeks after sowing the seeds). The R-
 59 value indicates the regularity of the spatial network, with lower values corresponding with more
 60 clustered, and higher values with more regular configurations

61



62

63

64 Figure S4: Aboveground biomass (g, dry weight) of *L. perenne* plants (a) and *P. lanceolata* in week 9
 65 (b) and their biomass ratio (c) as a function of the regularity (unitless) of the initial *Agrostis stolonifera*
 66 plants' spatial configuration.