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Demographic quantification of carbon and nitrogen dynamics associated with root turnover in white clover

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1 **Demographic quantification of carbon and nitrogen dynamics**
2 **associated with root turnover in white clover**

3

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15 Running title: Clover root C and N dynamics

16

17 **Abstract**

18 As well as capturing resources, roots lose resources during their lives. We quantified
19 carbon (C) and nitrogen (N) losses associated with root turnover in white clover
20 (*Trifolium repens* L.). We grew contrasting cultivars for 18 weeks in soil microcosms.
21 Using repeated in situ observations, destructive sampling, and demographic analysis,
22 we measured changes in C and N concentrations in dry matter of 1st- or 2nd-order
23 (terminal) roots to derive C and N fluxes into and out of root cohorts. C and N fluxes
24 from roots during turnover depended on cohort age and order. 90% of losses occurred
25 from 2nd-order cohorts younger than 18 weeks. Losses were greater from roots of the
26 larger, faster-growing cultivar Alice than from the smaller lower-yielding cultivar
27 S184. C:N ratios of roots and lost material were similar within each order and
28 between cultivars, but smaller in 2nd- compared with 1st-order roots. C and N losses
29 during root turnover could be equivalent to at least 6% of above-ground dry matter
30 production in S184 and 12% in Alice at the field scale. C and N losses associated with
31 root turnover will have potentially significant and previously unrecognised impacts on
32 crop productivity, resource dynamics and long-term soil fertility.

33

34 **Key words:** carbon, C and N loss, root turnover, growth, nutrients/nitrogen, *Trifolium*
35 *repens*

36

37

38 INTRODUCTION

39 Legumes have been included in low-input agricultural rotations for millennia. They
40 provide significant sources of forage, protein and oils, and maintain long-term soil
41 fertility mainly through the return to the soil of nitrogen (N)-rich crop residues at the
42 end of the growing season (Robson *et al.* 2002). A potentially important, yet poorly
43 understood, aspect of legume N dynamics is the loss from living plants of captured N.
44 Such losses occur during organ senescence or when plants are damaged by pests,
45 herbivores or extreme weather, but can also occur from healthy, living structures as
46 part of their normal metabolism.

47 Whatever their origin, the loss of N and other resources and their potential impacts on
48 productivity remain hard to quantify. This is especially true for losses from roots.
49 Analyses of leaf nutrients of many species has revealed that about half of the N in
50 leaves is lost from the plant during senescence, and the rest is retranslocated
51 internally; this also applies to most other nutrients (Robinson 2016). But no
52 comparably detailed information exists for the fate of nutrients in the roots of any
53 species.

54 A root imports resources as it grows. As the root ages and eventually senesces, some
55 or all of its contents will be lost to the soil, and an important input of new material to
56 soil organic matter, SOM (Rasmussen *et al.* 2010). The scale of that input will depend
57 on the absolute and relative amounts of carbon (C) and N gained and lost during a
58 root's life (Griffiths & Robinson 1992), and on the cumulative C and N fluxes through
59 all roots during the plant's life. The latter depend, in turn, on the dynamic
60 distributions of sizes, ages, longevities, phenologies and growth rates among the
61 components of the root system (Eissenstat & Yanai 1997; Guo *et al.* 2007; Goebel *et*
62 *al.* 2011; McCormack *et al.* 2015). Such distributions reflect the demography of the
63 root system.

64 Root demographic analyses involve repeated censuses of births, deaths, survival and
65 growth of identifiable members of a root system, information obtained non-
66 destructively using observation chambers, mini-rhizotrons, tomography, or magnetic
67 resonance imaging (Vetterlein & Doussan 2016). Root 'birth' is the emergence of a
68 new root from its parent; 'death' the disappearance of a root caused by senescence,

69 damage or herbivory; ‘survival’ is the time between root birth and death; and root
70 ‘growth’ is defined here as the progressive extension of a root in length and diameter.
71 Demographic approaches provide a wealth of information about the dynamic
72 behaviour of root structures (Gill & Jackson 2000 and references therein). But there is
73 scant information about how that behaviour relates to associated C and N fluxes. For
74 example, Hendrick & Pregitzer (1993) estimated annual total N, but not C, fluxes
75 during fine-root turnover in sugar maple (*Acer saccharum*). Pregitzer *et al.* (1997)
76 measured C and N concentrations in roots of different order in tree (*A. saccharum* and
77 *Fraxinus americana*) and forb (*Hydrophyllum canadense* and *Viola pubescens*)
78 species, but reported no temporal dynamics. Ruess *et al.* (2003) measured fine-root
79 dynamics in an Alaskan black spruce (*Picea mariana*) forest, focusing on how root
80 turnover related to *in vitro* respiration, rather than *in situ* C and N dynamics. The
81 conclusion reached by Ruess *et al.* that “The fate of fine-root C and N following root
82 disappearance remains a key question in the dynamics of C and element cycling”,
83 remains valid.

84 Our objective here was to measure C and N fluxes associated with the production,
85 growth and death of roots within intact root systems of white clover (*Trifolium repens*
86 L.), one of the most important legumes of temperate managed grasslands (Abberton &
87 Marshall 2005), and to relate these to potential impacts on crop productivity. To meet
88 these objectives we used a novel approach that combined sequential sampling and
89 chemical analyses of root tissues along with simultaneous root demography. We
90 aimed to answer four questions: (1) How much C and N are present in white clover
91 roots of different age and developmental order in intact, soil-grown root systems? (2)
92 How do those amounts of C and N change as a root system develops and as root
93 cohorts age? (3) How much C and N is lost from a root system when a root cohort
94 dies? (4) What are the potential implications of such losses for crop productivity?

95

96 **METHODS**

97 **Experimental requirements**

98 To estimate C and N fluxes associated with root turnover, sequential destructive
99 sampling is required to provide material for chemical analysis of roots alongside

100 demographic information obtained non-destructively. To meet these conflicting needs,
101 we used plants grown in soil rhizotrons. This allowed direct observation and detailed
102 tracking of individual roots within whole, intact root systems during censuses, as well
103 as destructive harvesting for the recovery of roots of known position and
104 developmental order for C and N analysis.

105 **Plant material and growing conditions**

106 Two white clover (*Trifolium repens* L.) cultivars (S184 and Alice) were compared.
107 Both have been recommended for commercial use in the UK. Alice is a fast-growing,
108 large-leaved, high-yielding cultivar. S184 is smaller-leaved and lower yielding.
109 Annual aboveground dry-matter yields of Alice averaged 4.0 t ha⁻¹ in field trials; those
110 of S184 were 2.5 t ha⁻¹ (Gilliland 2004). On that basis, we expected that C and N
111 losses from the higher-yielding cultivar Alice would exceed those from S184.
112 Perennial ryegrass swards containing Alice or S184 have similar above-ground
113 phenologies from Spring to Autumn (Gilliland 2004).

114 Plants were grown individually, from seed, for 18 weeks in flat glass-walled
115 rhizotrons. Each rhizotron was 61 cm deep × 30 cm wide × 1.5 cm thick, providing a
116 soil volume of 2.7 L at a bulk density of about 1.5 g cm⁻³, at the upper end of the
117 range for heavily grazed pastures (Van Haveren 1983; Davies *et al.* 1989). Further
118 details are in Scott *et al.* (2005).

119 Thirty rhizotrons, 15 for each cultivar, were packed with sieved pasture soil from
120 Craibstone, Aberdeenshire, UK (Countesswells soil association, derived from humus-
121 iron podzol overlying granitic rock) in a 1:1 w/w mixture with sand to improve
122 drainage. Rhizotrons were held at an angle of 20° to the vertical to encourage roots to
123 track the rear inner surface of the glass wall. Water was initially provided at 50 mL
124 per rhizotron every second day, sufficient to maintain field capacity. Irrigation was
125 increased to match plant demand during the experiment. All rhizotrons were
126 maintained in the same controlled-environment chamber (Conviron, Winnipeg,
127 Canada) with a 14 h photoperiod with a 20°C/10°C day/night regime. Fluorescent and
128 incandescent bulbs provided PAR at 500 μmol m⁻² s⁻¹. Each rhizotron was enclosed in
129 a light-proof baffle to shield soil and roots.

130

131 **Non-destructive root censuses**

132 During root censuses, baffles were removed and rhizotrons scanned at 300 dpi on an
133 A3-size flatbed scanner (Epson 836XL), calibrated for compatibility with
134 WinRHIZOTron™ software (Régent Instruments, Québec, Canada). Twenty-four bit
135 colour images were saved as uncompressed TIFF files. If root systems extended
136 below 40cm, the upper 40cm and lower 20cm sections of the rhizotron were scanned
137 separately, the images joined using Adobe Photoshop™. Sequential images of the
138 same root system were traced using the manual tracing function of WinRHIZOTron™.
139 When a new scanned image was analysed, the previous image of the same root system
140 was overlaid on it. All roots were numbered uniquely as discrete ‘paths’ such that
141 each new root was subsequently tracked as it extended and for as long as it survived.
142 The position, length and diameter of each root was traced and recorded. Growth rates
143 of existing roots were also recorded, as were root births. Roots or parts of roots that
144 disappeared between one time point and the next were classed as dead.

145 Non-destructive census data were obtained weekly for each rhizotron. But, to provide
146 sufficient root material for C and N analysis (see below), the minimum possible
147 interval for destructive sampling was three weeks. Therefore, weekly root censuses
148 were accumulated into 3-week intervals to match that to which the C and N data were
149 constrained.

150 Following a widely used developmental ordering scheme (Rose 1983; cf. topological
151 ordering e.g., Fitter 1986), we defined roots arising from the base of the stem as 1st-
152 order roots, and those arising from 1st-order roots as 2nd-order roots; the latter were the
153 finest, terminal branches as no 3rd-order roots were observed. This approach allowed
154 us to distinguish the behaviour of roots according to their age and developmental
155 origin. By contrast, most literature references to ‘fine-roots’ refer to all roots < 2 mm
156 diameter, irrespective of their age or developmental order (Wells & Eissenstat 2001;
157 Pregitzer, 2002; Guo *et al.* 2008). Note that some developmental ordering schemes
158 (e.g., McCormack *et al.* 2015) define all terminal fine-roots as 1st-order irrespective of
159 their time of appearance, a convention that re-orders roots whenever a new branching
160 level arises.

161 Output was generated as spreadsheets in which each row contained data for each
162 numbered root including its order, diameter, length, start and end positions (as 2D
163 spatial coordinates) and whether it was alive or dead. Roots produced during the first
164 3-week period were classified as belonging to “cohort **3**”; roots produced between 3-6
165 weeks belonged to “cohort **6**”; and so on for each 3-week interval. Accordingly, there
166 were no cohorts numbered **1, 2, 4, 5**, etc. The total root length of each cohort at each
167 census was calculated, as were changes in length between successive censuses caused
168 by births and deaths.

169 **Destructive harvesting**

170 Every three weeks, five replicate rhizotrons of each cultivar were harvested. The rear
171 glass panel was removed. Roots were excised using scalpel and tweezers, and any
172 adhering soil removed. Excised roots were combined into batches according to their
173 age (cohort) and order. The age and order of roots excised at the time of harvest was
174 determined by reference to scanned images (see above). For example, a 2nd-order root
175 born between weeks 3 and 6 was designated as “2nd-order, cohort **6**”; after 18 weeks
176 plant growth, that root would therefore be between 12 and 15 weeks old. Once
177 identified on screen, the root was then located within the rhizotron (unless the root
178 had died), excised and batched for analysis with other roots of similar order and
179 cohort harvested from that plant. Oven-dry weights of root batches were recorded (\pm
180 0.1 mg) after drying (60°C for 24 h). Specific root lengths (λ ; m g⁻¹) of each batch
181 were derived by dividing total length by dry weight. Total C and N concentrations (%
182 or mg g⁻¹) in the dry matter of replicate batches were determined by isotope ratio mass
183 spectrometry for which minimum sample dry weights of 1 mg were needed. Total C
184 and N contents per unit root length (mg m⁻¹) were calculated by dividing
185 concentrations by λ .

186 **Estimating C and N fluxes demographically**

187 The data used as inputs to the root demography calculations were, for each root cohort
188 and order, the C and N contents per unit root length as determined from destructive
189 sampling, and the lengths of existing, new and disappeared roots at each 3-week
190 interval estimated from censuses.

191 [Table 1 here]

192 Root C and N dynamics were calculated by adapting standard life-table analysis from
193 population biology (Begon *et al.* 1996, Ch. 1), but using quantities of C and N, rather
194 than numbers of individuals, in successive cohorts. This allows ‘balance sheets’ for C
195 and N in root structures to be calculated as successive cohorts are produced, grow and
196 senesce (Table 1). The logic of this scheme is that a root can pass from one age class
197 to the next, undergoing little physiological change, its C and N remaining within its
198 tissues. As an existing root extends, it imports C and N internally via its vascular
199 system or, in the case of N, by uptake from the soil, to support its growth. This
200 constitutes a gain in resources by that root, reflected as an increase in C and N
201 contents. When a root senesces or dies, some of its gained C and N are lost, as
202 indicated by a reduction in the cohort’s C or N content from the previous census.
203 These steps occur simultaneously. The calculations rest on several assumptions:

- 204 (1) Roots are populations of individuals grouped into cohorts produced at discrete 3-
205 week intervals. A root assigned to cohort **3**, for example, was produced within the
206 first 3 weeks of plant growth.
- 207 (2) Soil contamination of small root samples was negligible. Although we did not
208 check this directly, root samples were cleaned scrupulously and our calculations
209 suggest that even if up to one-tenth of a sample’s dry weight comprised
210 contaminating soil, C and N determinations would still have been within 2% of
211 those reported below.
- 212 (3) C and N losses by rhizodeposition, volatilisation or exudation (Paynel *et al.* 2001;
213 Jones *et al.* 2004; Sierra & Desfontaines 2009) were negligible relative to those
214 attributable directly to root turnover.
- 215 (4) C lost from roots by respiration (Ruess *et al.* 2003) was ignored, but was not
216 negligible. The relationship between root respiration and longevity is complex,
217 involving variable rates of consumption of recently assimilated and stored C
218 pools (Lynch *et al.* 2013). Respiration-derived C losses will add variable, but
219 unknown, amounts to our estimates of C losses associated with the turnover of
220 root structures.
- 221 (5) No internal retranslocation of C or N before root death occurred. Any such
222 retranslocation would be a net gain by (or reduced loss from) the plant. The evidence
223 suggests that for N the amounts are negligible (Gordon & Jackson 2000).

- 224 (6) Roots visible against the glass wall were representative of the entire root system
225 (Nagel *et al.* 2013).
- 226 (7) Root herbivory was negligible. Root-feeding nematodes would have been present
227 in the field soil that we used, but distributed equally across rhizotrons. No other
228 major root herbivores such as leatherjackets (Tipulidae) were observed.
- 229 (8) Plants grew normally in the rhizotrons compared with the field. This is unlikely to
230 have been strictly true, a failing that our experiment shares with others in which
231 roots are confined to less soil than they would have access to in the field (Poorter
232 *et al.* 2016). It would have been impossible to obtain the information we needed
233 using any other system. A rhizotron will always be a compromise, one that
234 nevertheless remains an essential tool in *in situ* root studies (Nagel *et al.* 2013).

235 Collectively, these assumptions mean that the estimated fluxes were probably
236 *minimum* amounts of C and N transferred within root cohorts as they aged, or that
237 were lost from the roots to the soil when they died. These, however, are the C and N
238 fluxes associated with the growth and replacement of root structures within the root
239 system, the specific targets of this study.

240 **Statistical analyses**

241 Effects of cultivar, root age and root order on variations in total C and N
242 concentrations and on specific root length (λ) were tested using General Linear
243 Models (GLMs) in Minitab (Minitab Inc.). λ data were ln-transformed to homogenize
244 variances. Interactions between cultivar, root order or root age were included in the
245 GLMs, but none were detected. ‘Rhizotron’ was included as a random factor. Models
246 were refined further based on the experiment’s power to detect genuine effects given
247 the degrees of freedom and with the false discovery rate set to 0.01 (Colquhoun
248 2014). This indicated that the appropriate *P*-value below which the effect of a factor
249 should be considered statistically ‘significant’ was $P = 0.002$, a far more rigorous
250 criterion than the conventional $P = 0.05$.

251

252

253 **RESULTS**

254 **Structural detail possible with rhizotron imaging**

255 [Fig. 1 here]

256 The structural detail provided by sequentially scanning entire root systems of white
257 clover is illustrated in Fig. 1. By 18 weeks, a root system of Alice typically comprised
258 over 2000 surviving 1st- and 2nd-order roots, representing a 40-fold net increase in root
259 number since week 3. No 3rd-order roots were present, despite the illusion that some
260 can be seen in Fig. 1; these were caused by minor software artefacts generated during
261 image overlay.

262 **Root C and N concentrations and specific root lengths**

263 [Table 2 here]

264 C and N concentrations in root dry matter were influenced most strongly by root order
265 (Table 2). In both cultivars, C concentrations were smaller in 2nd- compared with 1st-
266 order roots, averaging $31.1 \pm 0.55\%$ in 1st-order and $25.2 \pm 0.82\%$ in 2nd-order; mean
267 N concentrations varied likewise: $1.79 \pm 0.06\%$ in 1st-order; $1.64 \pm 0.08\%$ in 2nd-
268 order. C concentration also depended on root age, accounted for largely by the notably
269 smaller C concentrations in most 3-week-old roots compared with those of other ages,
270 especially in S184.

271 The mean root C concentrations of the two cultivars averaged over the two root orders
272 were statistically indistinguishable: $29.2 \pm 0.643\%$ in Alice and $27.7 \pm 0.702\%$ in
273 S184, as were the corresponding values for N concentration: $1.73 \pm 0.07\%$ and $1.71 \pm$
274 0.07% .

275 Root order was also the only influence on specific root length. λ averaged 97.0 ± 5.59
276 m g^{-1} in 1st-order roots and $241.0 \pm 19.1 \text{ m g}^{-1}$ in 2nd-order roots, respectively. This
277 implies smaller diameters in 2nd-order roots, as expected of terminal members of a
278 hierarchical branching system.

279 The coefficients of variation of root C and N concentrations and λ were c. 25%
280 overall. This indicates the typical variation that could be expected on the C and N
281 fluxes derived below using the scheme outlined in Table 1.

282 **Root C and N dynamics**

283 [Table 3 here]

284 Most C turnover in the root system of the larger cultivar, Alice, during 18 weeks of
285 plant growth occurred in the 2nd-order roots: 3.7-times as much C was lost from those
286 roots compared with from 1st-order roots (Table 3). The amount of C accumulated in
287 the dry matter of 2nd-order roots exceeded that in the 1st-order roots by 1.7-fold.

288 Unsurprisingly, most C loss associated with root turnover occurred towards the end of
289 the experiment as roots aged, but the oldest roots (cohort **3**) did not contribute most of
290 that loss. Cohorts **6**, **9** and **12** accounted for at least 92% of the total C lost in both
291 cultivars because those were the largest cohorts, produced when the root system was
292 growing exponentially.

293 Similar temporal patterns of C gain and turnover-related loss occurred in the smaller-
294 leaved cultivar, S184. Most C loss again occurred from the 2nd-order roots whose
295 losses were 1.6-times greater than from the 1st-order roots (Table 3). Unlike Alice,
296 however, S184 accumulated twice as much C in 1st- compared with 2nd-order roots:
297 672 and 312 mg C, respectively. Proportionally less gained C was lost from the roots
298 of S184 than from Alice, only 8.3 and 2.4% from the 2nd- and 1st-order roots,
299 respectively.

300 [Table 4 here]

301 Alice invested 76.7 mg N in root biomass over 18 weeks of growth; 2nd-order roots
302 received 51.0 mg, and 1st-order 25.7 mg (Table 4). The patterns of N loss by root
303 turnover in 2nd- and 1st-order roots of Alice were similar to those for C. Over 18
304 weeks, 7.2 mg N were lost from 2nd-order roots and 1.5 mg from 1st-orders. S184
305 invested 57.3 mg N in root biomass over 18 weeks; 2nd-order roots received 21.1 mg
306 N, less than the 36.2 mg N invested in 1st-order roots. Although 1st-order roots
307 contained more N than 2nd-orders, the latter lost more N.

308 Most investment of C and N in new root cohorts occurred during the first three weeks of
309 a cohort's existence, with one exception: in cohort 6 of Alice, more C, 72.3 mg, was
310 used to produce 1st-order roots between 3-6 weeks old than the 59.0 mg in the 0-3 week-
311 old roots of 6-week-old plants (Table 3). Typically, after the initially large investment,
312 each 1st- or 2nd-order root cohort lost more C and N by root turnover than it gained by
313 growth during each 3-week period. The successive production of younger cohorts
314 ensured that in the root system as a whole, C and N gains by growth exceeded C and N
315 losses by turnover. Losses were distributed unevenly between 1st- and 2nd-order roots.
316 Greater proportional losses occurred from 2nd-order roots than from 1st-order. Mean C
317 and N losses were 14% from 2nd-order roots of Alice compared with about 6% from 1st
318 orders; the corresponding figures for S184 were 8 and 2%, respectively. C and N losses
319 from S184 were proportionally smaller than those from Alice.

320 [Table 5 here]

321 The detailed demographic information in Tables 3 and 4 was combined to estimate the
322 C:N ratios of roots and of material gained by roots during growth and lost during turnover
323 (Table 5). The most notable features of Table 5 are: (a) the temporal stability of the C:N
324 ratios of roots within each order; (b) the similarity of root C:N ratio between the two
325 cultivars for roots in the same order; and (c) the similarity between mean C:N ratios of
326 roots and of material lost from them.

327 [Fig. 2 here]

328 The amounts of C and N gained on a whole-plant basis by the cohorts of 1st- and 2nd-
329 order roots of Alice amounted to 1218 mg C and 76.7 mg N during 18 weeks of plant
330 growth; the corresponding figures for S184 were 984 mg C and 57.3 mg N (Fig. 2).
331 The corresponding C and N losses from root turnover between weeks 3 and 18
332 totalled 134 mg C and 8.5 mg N from the roots of Alice, and 42.2 mg C and 2.3 mg N
333 from the roots of S184. These figures align with our expectation that losses from the
334 higher yielding cultivar Alice would exceed those from the smaller S184.

335

336

337 **DISCUSSION**

338 **C and N dynamics associated with root turnover**

339 Our data show clear and considerable differences in the potential for C and N transfer
340 to soil as a result of root turnover in white clover. Absolute and relative amounts of C
341 and N transferred to soil during root turnover in white clover varied with respect to
342 root age (i.e., cohort) and developmental order. Genetic differences were also
343 apparent in that C and N fluxes were greater from the roots of larger, faster-growing
344 cultivar Alice than from the smaller lower-yielding cultivar S184 grown under the
345 same conditions.

346 Most C and N loss arose from the turnover of 2nd-order roots (Tables 3 and 4). This is
347 strong evidence that terminal roots, the developmentally youngest and most
348 ephemeral members of the root system, account for a disproportionately large fraction
349 of the plant's dynamic interactions with surrounding soil, particularly the transfer into
350 the rhizosphere of C, N and other root contents. Terminal roots have been long-
351 suspected as having that function (Pregitzer 2002), but convincing evidence for it had
352 previously proved elusive.

353 An obvious difference between the white clover plants used in our experiments and
354 their field-grown counterparts is that the latter would be periodically cut or grazed.
355 Defoliation increases root turnover in some pasture species, but not white clover (Reid
356 *et al.* 2015). It is likely that the turnover rates we measured in undefoliated plants
357 would be uninfluenced by cutting.

358 If the data in Tables 3 and 4 are generally applicable, genotypes with greater turnover,
359 especially of terminal roots, will be needed for the effective management of grassland
360 swards to increase long-term C sequestration (Rees *et al.* 2005; Marshall *et al.* 2016).
361 Genotypes with greater root turnover, and therefore C and N deposition, at depth will
362 also be needed to minimise the risk of plant-derived labile C being rapidly converted
363 to CO₂ in surface soil and lost to the atmosphere. Developing white clover genotypes
364 with beneficial root traits has considerable potential (Caradus & Woodfield 1998;
365 Abberton & Marshall 2005) although, historically, breeding programmes have
366 focused on maximising aboveground production and forage quality. Marshall *et al.*
367 (2016) argue persuasively that this focus needs to encompass belowground traits to

368 fully realise the environmental and economic potential of managed grass-legume
369 swards. The development of automated, non-destructive phenotyping tools for this
370 purpose (Nagel *et al.* 2012; Marshall *et al.* 2016) is invaluable provided that they
371 accurately quantify the finest, most ephemeral roots within even the largest root
372 systems.

373 **Technical issues**

374 Like all sampling-based approaches, root demography has its strengths and
375 weaknesses (Sturite *et al.* 2007). One of the most fundamental but neglected sources
376 of variation is the interval between successive censuses. If the interval is too long
377 relative to turnover rate, growth and death rates of individual roots will be under-
378 estimated. For example, Stewart & Frank (2008) found that root growth and mortality
379 rates in upland grassland when estimated monthly using mini-rhizotrons were less
380 than half of those estimated when observations were separated by only 3 d, an interval
381 short enough to detect the dynamics of the most ephemeral roots. Based on a 3-week
382 census interval, imposed by the requirements of chemical analysis (see Methods), our
383 data showing that 2nd-order roots made the largest contribution to the loss of root C
384 and N from white clover root systems could be under-estimates. The scale of the
385 contributions of such roots to root C and N dynamics could be even larger than our
386 data indicate.

387 Direct estimates of the amounts of C and N lost from entire root systems of clover
388 have been obtained using *in situ* isotope (¹⁴C, ¹⁵N) labelling (e.g., Rasmussen *et al.*
389 2007). Isotopically estimated losses and transfers to neighbouring plants reflect the
390 net effects of all the turnover, exudation and rhizodeposition processes in the whole
391 root system between labelling and harvest. What isotopic approaches cannot do is to
392 distinguish the contributions of developmentally distinct parts of the root system (e.g.,
393 1st- versus 2nd-order roots; Guo *et al.* 2008); nor can they separate the effects of root
394 turnover *per se* from other processes (Kuzyakov & Domanski 2000). To fully
395 appreciate how the interplay between physiology, developmental morphology and
396 demography controls such fluxes it is necessary to sample and analyse roots according
397 to their order in the branching hierarchy and not to assume functional homogeneity
398 throughout the root system (Valenzuela-Estrada *et al.* 2008; Rasmussen *et al.* 2010;

399 Goebel *et al.* 2011; Vetterlein & Doussan 2016), and to then to scale up information
400 obtained at the individual-root level to that of the whole system.

401 **Scaling to seasonal effects**

402 Our 18-week experiment was sufficiently long to capture the detailed root dynamics
403 of white clover plants up to that age, a period coinciding with that of maximum rates
404 of vegetative growth and resource capture of temperate clover crops (Black 1957;
405 Silsbury 1984). Obviously, C and N fluxes associated with root turnover throughout
406 that period would be dwarfed by those occurring when legume crop residues are
407 ploughed into soil at the end of the growing season which, for white clover in
408 temperate regions, typically lasts 20-25 weeks (Rasmussen *et al.*, 2013). Even so,
409 Rasmussen *et al.* (2013) concluded that short-term N fluxes from clover roots could
410 also make significant contributions to N budgets of grass-clover swards. Our data
411 show that N loss rates are not constant across the root system nor through time during
412 the vegetative growth of white clover. Moreover, there is likely to be genetic variation
413 in N fluxes if the comparison between Alice and S184 indicates a general association
414 between root N loss and potential productivity, and if our findings can be translated to
415 field settings.

416 A possible issue that we have not investigated here is that of phenological differences
417 between cultivars, and their influences on root C and N loss. Any phenological
418 differences between cultivars would have been detected by the sequential sampling
419 (cf. experiments comprising only one final harvest: Trinder *et al.*, 2012). The data in
420 Tables 3 & 4 suggests no obvious cultivar difference in the phenology of root C or N
421 losses during the experiment. But over an entire annual cycle it is possible that
422 cultivar differences in the timing of root-derived C and N inputs to soil could occur.

423 The longevity of white clover roots is enormously variable. Estimates of mean or
424 median lifespans ranging from 1-6 (Watson *et al.* 2000), 15 (Reid *et al.* (2015), 4-37
425 (Harper *et al.* 1991) and 40 weeks (Sturite *et al.* 2007) have been reported. This
426 variation mainly reflects seasonal and geographic influences. Greater and more rapid
427 root mortality of the white clover cultivar S184 occurred at a warmer site in Italy than
428 at a colder UK site (Watson *et al.* 2000). Sturite *et al.* (2007) reported a strong linear
429 decline in median longevity of white clover roots as soil temperatures increased.

430 Whether warmer soil results in the loss of more or less C and N via root turnover will
431 depend on the balance between faster root growth and more rapid mortality. If soil
432 warming accelerates the latter more than the former, C and N losses will probably
433 increase; if warming increases growth more than death, losses should decrease. But
434 the temperature responses of root demographics can be transient and are influenced
435 indirectly by temperature-related changes in nutrient availability, at least in temperate
436 grasslands (Fitter *et al.* 1999; Edwards *et al.* 2004). It would be valuable to apply the
437 demographic approach reported here to directly test the effects of temperature and
438 other factors on root C and N dynamics to clarify the extent to which they are
439 environmentally constrained.

440 **Implications for crop productivity**

441 N lost from a legume's root system can be equated notionally to a potential productivity
442 'loss' for that crop, it might also equate to a gain for the next crop in the rotation if it
443 can take advantage of that N. Likewise, C lost from a root system cannot contribute
444 directly to the productivity of that crop but, as SOM, might sustain the productivity of
445 subsequent crops (Rasmussen *et al.* 2010) or contribute to long-term C sequestration
446 (Rees *et al.* 2005; Marshall *et al.* 2016).

447 To scale up the C and N losses per plant (Fig. 2) to estimate potential effects on field
448 crops, we assumed a typical planting density of 100 m⁻² (Marshall & James 2006). The
449 estimated mean weekly C and N losses by root turnover over 18 weeks' growth would
450 have been equivalent to 7.5 and 0.5 kg ha⁻¹ for Alice and 2.3 and 0.1 kg ha⁻¹ for S184,
451 respectively. If total above-ground dry matter production was 4.0 t ha⁻¹ for Alice and 2.5 t
452 ha⁻¹ for S184 (Gilliland 2004) and mean cultivar-specific C and N concentrations in dry
453 matter those reported in Table 2, total C and N losses from the roots of Alice would be
454 about 134 and 8.5 kg ha⁻¹, respectively; corresponding figures for S184 are 42.2 and 2.3
455 kg ha⁻¹.

456 The C and N losses we estimated for white clover are, therefore, equivalent to about 6%
457 of above-ground dry matter production of the slower-growing cultivar S184 and up to
458 12% of that of the higher-yielding cultivar Alice. The plausibility of these estimates is
459 supported by independent evidence. Using extensive isotope labelling data, Kuzyakov &
460 Domanski (2000) suggested that annual root-derived C fluxes (including root turnover,

461 exudation, rhizodeposition and other processes, but excluding respiration) into pasture
462 soil averages about 7% of total aboveground dry matter production. The similarity of this
463 figure to the 6-12% we estimated for C and N loss solely via root turnover hints that the
464 bulk of such fluxes does indeed originate from root turnover, and that exudation and
465 similar processes make negligible contributions at the field scale (see assumption (3) in
466 Methods).

467 Even so, 6-12% might appear to be trivial fractions of potential crop productivity, given
468 the much larger variations caused by unpredictable weather or heterogeneous soil
469 conditions (Wilman *et al.* 2005; Frankow-Lindberg *et al.* 2009; Lobell *et al.* 2009). But
470 we again emphasise that ours are conservative estimates of C and N losses associated
471 only with root turnover and, therefore, of the potential of that process to reduce notional
472 productivity, and are estimated for only an 18-week period. Consequently, it is likely that
473 the constraint on potential productivity attributable to root turnover will exceed our
474 estimates. It is more complicated than that, however, because accumulated crop-derived
475 C and N inputs influence soil conditions that can modify future productivity (e.g., N
476 availability, SOM composition). Therefore, it is equally possible that any potential losses
477 in clover productivity caused by root turnover could be offset in the long-term by
478 improved soil fertility that will benefit a subsequent crop in the rotation.

479 **CONCLUSIONS**

480 The detailed information reported here provides a new perspective on C and N dynamics
481 associated with root turnover in an agriculturally important legume. Using a novel
482 approach combining non-destructive root censuses with sequential destructive sampling,
483 and demographic modelling, we have estimated that C and N fluxes associated with root
484 turnover in white clover represent a potential loss in crop productivity of at least 6-12%.
485 Those fluxes were not distributed evenly over whole root systems, but arose mainly from
486 the turnover of relatively young, ephemeral terminal members of the root system. There
487 is likely to be significant genetic variation in the contributions of white clover to soil
488 fertility and potential C sequestration via root-derived C and N inputs.

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494

495 REFERENCES

- 496 Abberton M.T. & Marshal A.H. (2005) Progress in breeding perennial clovers for
497 temperate agriculture. *Journal of Agricultural Science*, 143, 117-135.
- 498 Begon M., Mortimer M. & Thompson D.J. (1996) *Population ecology. A unified study*
499 *and animals and plants*. 3rd ed. Blackwell Science Ltd, Oxford, UK.
- 500 Black J.N. (1957) Seed size as a factor in the growth of subterranean clover (*Trifolium*
501 *subterraneum* L.) under spaced and sward conditions. *Australian Journal of*
502 *Agricultural Research* 8, 335-351.
- 503 Caradus J.R. & Woodfield DR. (1998) Genetic control of adaptive root characteristics in
504 white clover. *Plant and Soil* 200, 63–69.
- 505 Colquhoun D. (2014) An investigation of the false discovery rate and the
506 misinterpretation of *p*-values. *Royal Society of London Open Science* 1, 140216
507 doi.org/10.1098/rsos.140216.
- 508 Davies A., Adams W.A. & Wilman D. (1989) Soil compaction in permanent pasture
509 and its amelioration by slitting. *Journal of Agricultural Science* 113, 189-197.
- 510 Edwards E.J., Benham D.G., Marland L.A. & Fitter A.H. (2004) Root production is
511 determined by radiation flux in a temperate grassland community. *Global Change*
512 *Biology* 10, 209-227.
- 513 Eissenstat D.M. & Yanai R.D. (1997) The ecology of root lifespan. *Advances in Ecological*
514 *Research* 27, 2-59.
- 515 Fitter A.H. (1986) The topology and geometry of plant-root systems, influence of watering
516 rate on root-system topology in *Trifolium pratense*. *Annals of Botany* 58, 91-101.
- 517 Fitter A.H., Self G.K., Brown T.K., Bogie D.S., Graves J.D., Benham D.G. & Ineson
518 P. (1999) Root production and turnover in an upland grassland subjected to
519 artificial soil warming respond to radiation flux and nutrients, not temperature.
520 *Oecologia* 120, 575-581.
- 521 Frankow-Lindberg B.E., Halling M., Höglind M. & Forkman J. (2009) Yield and
522 stability of yield of single- and multi-clover grass-clover swards in two contrasting
523 temperate environments. *Grass and Forage Science* 64, 236-245.

524 Gilliland T.J. (2004) *Grass and clover. Recommended varieties for Northern Ireland*
525 (2004)/05. Department of Agriculture and Rural Development, Belfast, UK.

526 Goebel M., Hobbie S.M., Bulaj B., Zadworny M., Archibald D.D., Oleksyn J., Reich
527 P.B. & Eissenstat D.M. (2011) Decomposition of the finest root branching orders,
528 linking belowground dynamics to fine-root function and structure. *Ecological*
529 *Monographs* 81, 89-102.

530 Gordon W.S. & Jackson R.B. (2000) Nutrient concentrations in fine roots. *Ecology*
531 81, 275-280.

532 Griffiths B. & Robinson D. (1992) Root-induced nitrogen mineralization, a nitrogen-
533 balance model. *Plant and Soil* 139, 253-263.

534 Guo D., Li H., Mitchell R.J., Han W., Hendricks J.J., Fahey T.J. & Hendrick R.L.
535 (2008) Fine root heterogeneity by branch order, exploring the discrepancy in root
536 turnover estimates between minirhizotron and carbon isotopic methods. *New*
537 *Phytologist* 177, 443-456.

538 Harper J.L., Jones M. & Sackville Hamilton N.R. (1991) The evolution of roots and
539 the problems of analysing their behaviour. In *Plant root growth. An ecological*
540 *perspective*. (ed D. Atkinson), pp. 3-22. Blackwell Scientific Publications, Oxford,
541 UK.

542 Hendrick R.L. & Pregitzer K.S. (1993) The dynamics of fine root length, biomass,
543 and nitrogen content in two northern hardwood ecosystems. *Canadian Journal of*
544 *Forest Research* 23, 2507-2520.

545 Jones D.L., Hodge A. & Kuzyakov Y. (2004) Plant and mycorrhizal regulation of
546 rhizodeposition. *New Phytologist* 163, 459-480.

547 Kuzyakov Y. & Domanski G. (2000) Carbon input by plants into the soil. Review.
548 *Journal of Plant Nutrition and Soil Science* 163, 421-431.

549 Lobell D.B., Cassman K.G. & Field C.B. (2009) Crop yield gaps, their importance,
550 magnitudes, and causes. *Annual Review of Environment and Resources* 34, 179-204.

551 Lynch D.J., Matamala R., Iversen C.M., Norby R.J. & Gonzalez-Meler M.A. (2013)
552 Stored carbon partly fuels fine-root respiration but is not used for production of
553 new fine roots. *New Phytologist* 199, 420-430.

554 Marshall A.H. & James I.R. (2006) Effect of plant density on stolon growth and
555 development of contrasting white clover (*Trifolium repens*) varieties and its
556 influence on the components of seed yield. *Grass and Forage Science* 43, 313-318.

557 Marshall A.H., Collins R.P., Humphreys M.W. & Scullion J. (2016) A new emphasis
558 on root traits for perennial grass and legume varieties with environmental and
559 ecological benefits. *Food and Energy Security* 5, 26-39.

560 McCormack M.L., Dickie I.A., Eissenstat D.M., Fahey T.J., Fernandez C.W., Guo D.,
561 ..., Zadworny M. (2015) Redefining fine roots improves understanding of below-
562 ground contributions to terrestrial biosphere processes. *New Phytologist* 207, 505-
563 518.

564 Nagel K.A., Putz A., Gilmer F., Heinz K., Fischbach A., Pfeifer J., ..., Schurr U.
565 (2012) GROWSCREEN-Rhizo is a novel phenotyping robot enabling simultaneous
566 measurements of root and shoot growth for plants grown in soil-filled rhizotrons.
567 *Functional Plant Biology* 39, 891–904.

568 Paynel F., Murray P.J. & Cliquet B. (2001) Root exudates, a pathway for short-term N
569 transfer from clover and ryegrass. *Plant and Soil* 229, 235-243.

570 Poorter H., Fiorani F., Pieruschka R., Wojciechowski T., van der Putten W., Kleyer
571 M., Schurr U. & Postma J. (2016) Pampered inside, pestered outside? Differences
572 and similarities between plants growing in controlled conditions and in the field.
573 *New Phytologist* 212, 838–855.

574 Pregitzer K.S. (2002) Fine roots of trees – a new perspective. *New Phytologist* 154,
575 267-273.

576 Pregitzer K.S., Kubiske M.E., Yu C.K. & Hendrick R.L. (1997) Relationships among
577 root branch order, carbon, and nitrogen in four temperate species. *Oecologia* 111, 302-
578 308.

579 Rasmussen J., Eriksen J., Jensen E.S., Esben K.H. & Høgh-Jensen H. (2007) In situ
580 carbon and nitrogen dynamics in ryegrass–clover mixtures, transfers, deposition
581 and leaching. *Soil Biology and Biochemistry* 39, 804-815.

582 Rasmussen J., Eriksen J., Jensen E.S. & Høgh-Jensen H. (2010) Root size fractions of
583 ryegrass and clover contribute differently to C and N inclusion in SOM. *Biology
584 and Fertility of Soils* 46, 293-297.

585 Rasmussen J., Gylfadóttir T., Loges R., Eriksen J. & Helgadóttir A. (2013) Spatial
586 and temporal variation in N transfer in grass-white clover mixtures at three
587 Northern European field sites. *Soil Biology and Biochemistry* 57, 654-662.

588 Rees R.M., Bingham I.J., Baddeley J.A. & Watson C.A. (2005) The role of plants and
589 land management in sequestering soil carbon in temperate arable and grassland
590 ecosystems. *Geoderma* 128, 130-154.

591 Reid J.B., Gray R.A.J., Springett J.A. & Crush J.R. (2015) Root turnover in pasture
592 species, chicory, lucerne, perennial ryegrass and white clover. *Annals of Applied*
593 *Biology* 167, 327-342.

594 Robinson D. (2016) Constraints on nutrient dynamics in terrestrial vegetation. In *A*
595 *biogeoscience approach to ecosystems* (eds E.A. Johnson & Y.E. Martin), pp.
596 254-291. Cambridge University Press Cambridge, UK.

597 Robson M.C., Fowler S.M., Lampkin N.H., Leifert C., Leitch M., Robinson D.,
598 Watson C.A. & Litterick A.M. (2002) The agronomic and economic potential of
599 break crops for ley/arable rotations in temperate organic agriculture. *Advances in*
600 *Agronomy* 77, 369-427.

601 Rose D.A. (1983) The description of the growth of root systems. *Plant and Soil* 75,
602 405-415.

603 Ruess R.W., Hendrick R.L., Burton A.J., Pregitzer K.S., Sveinbjornsson B., Allen
604 M.F. & Maurer G.E. (2003) Coupling fine root dynamics with ecosystem carbon
605 cycling in black spruce forests of interior Alaska. *Ecological Monographs* 73, 643-
606 662.

607 Scott G.D., Baddeley J.A., Robinson D. & Watson CA. (2005) Portable rhizotron
608 system for digital sequencing and physical trait analysis of developing root
609 systems. *Aspects of Applied Biology* 73, 63-68.

610 Sierra J. & Desfontaines L. (2009) Role of root exudates and root turnover in the
611 below-ground N transfer from *Canavalia ensiformis* (jackbean) to the associated
612 *Musa acuminata* (banana) *Crop and Pasture Science* 60, 289-294.

613 Silsbury J.H. (1984) Comparison of the growth-rates of dinitrogen fixing subterranean
614 clover swards with those assimilating nitrate ions. *Plant and Soil* 80, 201-213.

615 Stewart A.M. & Frank D.A. (2008) Short sampling intervals reveal very rapid root
616 turnover in a temperate grassland. *Oecologia* 157, 453-458.

617 Sturite I., Henriksen T.M. & Breland T.A. (2007) Longevity of white clover (*Trifolium*
618 *repens*) leaves, stolons and roots, and consequences for nitrogen dynamics under
619 northern temperate climatic conditions. *Annals of Botany* 100, 33-40.

620 Trinder C.J., Brooker R., Davidson H. & Robinson D. (2012) Dynamic trajectories of
621 growth and nitrogen capture by competing plants. *New Phytologist* 193, 948-958.

622 Valenzuela-Estrada L.R., Vera-Caraballo V., Ruth L.E. & Eissenstat D.M. (2008)
623 Root anatomy, morphology, and longevity among root orders in *Vaccinium*
624 *corymbosum* (Ericaceae) *American Journal of Botany* 95, 1506-1514.

- 625 Van Haveren B.P. (1983) Soil bulk density as influenced by grazing intensity and soil
626 type on a shortgrass prairie site. *Journal of Range Management* 36, 586-588.
- 627 Vetterlein D. & Doussan C. (2016) Root age distribution, how does it matter in plant
628 processes? A focus on water uptake. *Plant and Soil* 407, 145-160.
- 629 Watson C.A., Ross J.M., Bagnaresi U., Minotta G.F., Roffi F., Atkinson D., Black
630 K.E. & Hooker J.E. (2000) Environment-induced modifications to root longevity in
631 *Lolium perenne* and *Trifolium repens*. *Annals of Botany* 85, 397-401.
- 632 Wells C.E. & Eissenstat D.M. (2001) Marked differences in survivorship among apple
633 roots of different diameters. *Ecology* 82, 882-892.
- 634 Wilman D., Oloms F. & Hamilton R.S. (2005) The potential of seed-shedding and
635 seedling development to contribute to the persistence of white clover (*Trifolium*
636 *repens*) in grazed swards in Uruguay. *Journal of Agricultural Science* 143, 493-501.
637

638 **Figure legends**

639

640 **Figure 1** Sequential digital tracing of the same root system of a *Trifolium repens* cv.
641 Alice individual at 3-week intervals over 18 weeks of plant growth. Each root path is
642 identified uniquely (green numbers on images). Tracings have been superimposed on
643 a black background for clarity. (a) Week 3, 58 root paths; (b) week 6, 179 paths; (c)
644 week 9, 727 paths; (d) week 12, 1302 paths; (e) week 15, 1674 paths; (f) week 18,
645 2299 paths.

646

647 **Figure 2** Summary of total C and N contained in root systems of two white clover
648 cultivars after 3 and 18 weeks' growth (numbers in boxes), and the net amounts lost
649 from the root system during 18 weeks' growth (numbers in arrows), derived from data
650 in Tables 3 and 4. a: Cv. Alice. b: Cv. S184.

651 **Table 1** Demographic scheme to calculate C or N dynamics of two root cohorts of a single root order.

Plant age	Root cohort						
	1			2			Total loss (mg per preceding time period)
	Mass	Gain	Loss	Mass	Gain	Loss	
1	X_1	$E_1 = QR_{1n}/\lambda$	$L_1 = QR_{1d}/\lambda$				
2	$X_2 = X_1 + E_1 - L_1$	$E_2 = QR_{2n}/\lambda$	$L_2 = QR_{2d}/\lambda$	Y_2	$F_2 = QR_{2n}/\lambda$	$M_2 = QR_{2d}/\lambda$	L_1
3	$X_3 = X_2 + E_2 - L_2$	$E_3 = QR_{3n}/\lambda$	$L_3 = QR_{3d}/\lambda$	$Y_3 = Y_2 + F_2 - M_2$	$F_3 = QR_{3n}/\lambda$	$M_3 = QR_{3d}/\lambda$	$L_2 + M_2$
							Total (mg)
Loss per cohort			$L_1 + L_2$			M_2	$L_1 + L_2 + M_2$
Mass per cohort	$X_1 + E_1 + E_2$			$Y_2 + F_2$			$X_1 + E_1 + E_2 + Y_2 + F_2$

652

653 This example shows the calculations for two root cohorts (denoted as **1** and **2**, which were formed by a plant at age 1 and between ages 1 and 2,
654 respectively) of the same developmental order. Fluxes of material into or out of root dry matter associated with growth or death are indicated as
655 Gain or Loss. X = mass (mg) of C or N in cohort **1**. E = C or N flux (mg) into cohort **1** caused by new root growth. L = C or N lost (mg) from
656 cohort **1** by root death. Y , F , M = corresponding values for cohort **2**. Subscripted numbers denote the plant age at which the flux occurred or to
657 which the masses of C or N apply. Q = C or N concentration (mg g^{-1}) in root dry matter; R = root length (m); subscripted letters ‘n’ and ‘d’
658 denote newly produced and dead root lengths, respectively; λ = specific root length (m g^{-1}) calculated separately for each cohort. (In this
659 example, fluxes subscripted 3, do not feature in the calculations because these would contribute to gains by and losses only from plants of age 4

660 and older.) Total losses during each preceding time interval (i.e., between plant harvests), summed for all cohorts, are calculated in the final
661 column. Total C or N masses in, and losses from, each cohort, and for all cohorts combined, are calculated in the final three rows. To
662 accommodate data for older plants and more root cohorts, this scheme is extended accordingly. C or N fluxes were derived separately for each
663 root order.

664 **Table 2** Cultivar-, order- and age-dependent variations in root C and N concentrations
 665 and specific root length (λ) of white clover from which C and N fluxes were derived.

Cultivar	Root order	Plant age (wk)	C (%)		N (%)		λ (m g ⁻¹)	
			mean	se	mean	se	mean	se
Alice	1	3	25.1	1.54	1.76	0.41	105.0	21.0
		6	32.3	1.44	1.88	0.14	109.2	21.0
		9	34.4	1.33	2.03	0.01	126.1	16.8
		12	34.9	1.54	1.95	0.08	88.2	10.1
		15	33.8	1.33	2.02	0.09	75.6	27.7
		18	34.6	1.64	1.71	0.11	21.0	0.67
	2	3	29.2	4.10	1.84	0.30	210.1	79.8
		6	25.6	1.03	1.65	0.12	208.4	67.2
		9	22.1	1.23	1.43	0.17	264.7	29.4
		12	25.1	1.85	1.31	0.16	214.3	33.6
		15	23.6	2.05	1.46	0.25	189.1	31.1
		18	-	-	-	-	-	-
S184	1	3	19.3	4.25	1.64	0.39	134.5	21.0
		6	27.7	1.78	1.77	0.15	168.1	18.5
		9	33.3	0.40	1.90	0.004	147.1	22.7
		12	32.1	0.30	1.54	0.01	100.8	12.6
		15	32.6	0.20	1.70	0.01	75.6	10.1
		18	33.1	0.15	1.55	0.01	12.6	0.42
	2	3	20.7	3.95	1.84	0.31	210.1	33.6
		6	27.5	1.19	2.29	0.14	247.9	33.6
		9	26.0	1.38	1.74	0.17	247.9	25.2
		12	26.7	1.58	1.44	0.17	357.1	96.6
		15	25.9	2.17	1.39	0.27	260.5	54.6
		18	-	-	-	-	-	-

Summary analysis of variance ^a

	d.f.	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Cultivar	1	2.38	0.125	4.3	0.04	1.16	0.287
Root order	1	60.61	<0.001	11.82	<0.001	61.47	<0.001
Plant age	4	7.86	<0.001	2.05	0.09	1.93	0.121
Error	164						

666 ^a Statistical effects of cultivar, root order and root age on total C and N concentrations
 667 (both symbolised as Q in Table 1) and λ , as determined by GLMs, are summarised as
 668 *F* ratios and *P* values; those in bold indicate $P \leq 0.002$, as explained in Methods. λ
 669 data were ln-transformed before analysis to homogenise variances. $n = 5$ throughout.

Table 3 Mean masses of C (mg) gained by, lost from, and contained in 1st- and 2nd-order root cohorts of two white clover cultivars of different ages.

Cultivar	Order	Plant age (weeks)	Root cohort number																
			3			6			9			12			15				
			Mass	Gain	Loss	Mass	Gain	Loss	Mass	Gain	Loss	Mass	Gain	Loss	Mass	Gain	Loss		
Alice	1	3	29.4	0.8	0.0												Loss (mg per 3 wk)		
		6	30.2	0.1	0.0	59.0	72.3	0.0										0.0	
		9	30.3	0.7	0.0	131.3	2.7	1.3	134.0	35.5	1.7								0.0
		12	31.0	0.0	0.0	132.7	0.5	0.5	167.8	1.1	2.6	80.8	4.4	2.2					3.0
		15	31.0	0.0	0.1	132.7	0.0	7.7	166.3	0.1	4.9	83.0	0.4	7.5	25.1	9.1	0.1		5.3
		18	30.9	-	-	125.0	-	-	161.5	-	-	75.9	-	-	34.1	-	-		20.3
			Loss (mg per cohort)		0.1		9.5		9.2		9.7						0.1	Total (mg)	
			Mass (mg per cohort)	31.0		135		171		85.6		34.2						28.5	
		2	3	7.8	0.4	0.3												Loss (mg per 3 wk)	
	6		7.9	0.0	1.3	60.3	5.2	1.3										0.3	
	9		6.6	0.0	1.8	64.2	0.0	12.4	238.0	34.8	3.8								2.6
	12		4.8	0.0	2.4	51.8	0.0	15.9	269.0	7.5	16.4	288.0	5.6	6.1					18.0
15	2.4		0.0	1.1	35.9	0.0	7.7	260.1	1.6	19.2	287.5	3.1	17.1	106.0	3.4	0.1		40.8	
18	1.3		-	-	28.2	-	-	242.5	-	-	273.5	-	-	109.3	-	-		45.2	
		Loss (mg per cohort)		6.9		37.3		39.4		23.2						0.1	Total (mg)		
		Mass (mg per cohort)	8.2		66		282		297		109						107		
S184	1	3	39.3	10.6	0.0												Loss (mg per 3 wk)		
		6	49.9	0.0	0.0	140.0	54.3	0.0										0.0	
		9	49.9	0.0	0.0	194.3	16.0	4.7	96.4	60.4	0.0							0.0	
		12	49.9	0.0	0.6	205.6	0.0	4.8	156.8	0.0	0.0	133.0	38.8	0.0				4.7	
		15	49.3	0.3	0.2	200.8	0.0	3.5	156.8	0.0	2.6	171.8	2.7	0.0	51.7	28.7	0.0		5.4
		18	49.4	-	-	197.3	-	-	154.2	-	-	174.5	-	-	80.4	-	-		6.3
			Loss (mg per cohort)		0.8		13		2.6		0						0.0	Total (mg)	
			Mass (mg per cohort)	50.2		210		157		175		80.4						16.4	
		2	3	2.6	0.0	0.0												Loss (mg per 3 wk)	
	6		2.6	0.1	0.1	33.0	2.8	0.1											0.0
	9		2.6	0.0	1.0	35.7	0.2	1.9	59.4	20.4	0.4								0.2
	12		1.6	0.0	0.4	34.0	0.0	5.8	79.4	0.9	2.5	96.6	7.4	1.2					3.3
15	1.2		0.0	0.2	28.2	0.0	4.7	77.8	0.2	5.4	102.8	0.5	1.8	72.5	14.9	0.3		9.9	
18	1.0		-	-	23.5	-	-	72.6	-	-	101.5	-	-	87.1	-	-		12.4	
		Loss (mg per cohort)		1.7		12.5		8.3		3.0						0.3	Total (mg)		
		Mass (mg per cohort)	2.7		36.0		80.9		105		87.4						25.5		
																	312		

Data were calculated according to the scheme shown in Table 1.

Table 4 Mean masses of N (mg) gained by, lost from, and contained in 1st- and 2nd-order root cohorts of two white clover cultivars of different ages.

Cultivar	Order	Plant age (weeks)	Root cohort number															Loss (mg per 3 wk)	
			3			6			9			12			15				
			Mass	Gain	Loss	Mass	Gain	Loss	Mass	Gain	Loss	Mass	Gain	Loss	Mass	Gain	Loss		
Alice	1	3	1.7	0.0	0.0													0.0	
		6	1.7	0.0	0.0	3.3	4.1	0.0										0.0	
		9	1.7	0.1	0.0	7.4	0.1	0.0	7.6	2.1	0.1							0.1	
		12	1.8	0.0	0.0	7.5	0.0	0.0	9.6	0.0	0.1	4.6	0.2	0.1				0.1	
		15	1.8	0.0	0.1	7.5	0.0	0.4	9.5	0.0	0.3	4.7	0.0	0.4	1.4	0.5	0.0	0.2	
		18	1.7			7.1			9.2			4.3			1.9			1.2	
			Loss (mg per cohort)															0.1	
			Mass (mg per cohort)	1.8			7.5			9.7		5			1.9			0.0	
																		1.5	
		2	3	0.5	0.0	0.0													0.0
6	0.5		0.0	0.1	4.1	0.5	0.1											0.0	
9	0.4		0.0	0.1	4.5	0.0	0.8	16.0	2.4	0.3								0.2	
12	0.3		0.0	0.2	3.7	0.0	1.1	18.1	0.5	1.1	19.3	0.4	0.4					1.2	
15	0.1		0.0	0.1	2.6	0.0	0.5	17.5	0.0	1.3	19.3	0.0	1.1	7.1	0.2	0.0		2.8	
18	0.0				2.1			16.2			18.2			7.3				3.0	
		Loss (mg per cohort)																0.5	
		Mass (mg per cohort)	0.5			4.6			18.9		19.7			7.3			0.0		
																		7.2	
																		51.0	
S184	1	3	2.1	0.6	0.0													0.0	
		6	2.7	0.0	0.0	7.6	2.9	0.0											0.0
		9	2.7	0.0	0.0	10.5	0.6	0.0	5.2	3.3	0.0								0.0
		12	2.7	0.0	0.0	11.1	0.0	0.3	8.5	0.0	0.0	7.2	2.1	0.0					0.0
		15	2.7	0.0	0.0	10.8	0.0	0.2	8.5	0.0	0.1	9.3	0.2	0.0	2.8	1.6	0.0		0.3
		18	2.7			10.6			8.4			9.5			4.4				0.3
			Loss (mg per cohort)																0.0
			Mass (mg per cohort)	2.7			11.1			8.5		9.5			4.4			0.0	
																			0.6
																			36.2
	2	3	0.2	0.0	0.0													0.0	
6		0.2	0.0	0.0	2.2	0.2	0.0											0.0	
9		0.2	0.0	0.1	2.4	0.0	0.1	4.0	1.4	0.0								0.0	
12		0.1	0.0	0.0	2.3	0.0	0.4	5.4	0.1	0.2	6.6	0.5	0.1					0.2	
15		0.1	0.0	0.0	1.9	0.0	0.3	5.3	0.0	0.4	7.0	0.0	0.1	4.9	1.0	0.0		0.7	
18		0.1			1.6			4.9			6.9			5.9				0.8	
		Loss (mg per cohort)																0.1	
		Mass (mg per cohort)	0.2			2.4			5.5		7.1			5.9			0.0		
																		1.7	
																		21.1	

Data were calculated according to the scheme shown in Table 1.

Table 5 C:N ratios of material gained by, lost from, and contained in 1st- and 2nd-order root cohorts of two white clover cultivars of different ages.

Cultivar	Order	Plant age (weeks)	Root cohort number															Mean ± s.e. (mg)			
			3			6			9			12			15						
			Mass	Gain	Loss	Mass	Gain	Loss	Mass	Gain	Loss	Mass	Gain	Loss	Mass	Gain	Loss				
Alice	1	3	17.3																		
		6	17.8			17.9	17.6														
		9	17.8	7.0		17.7	27.0		17.6	16.9	17.0										
		12	17.2			17.7			17.5		26.0	17.6	22.0	22.0							
		15	17.2			17.7		19.3	17.5		16.3	17.7		18.8	17.9	18.2					
		18	18.2			17.6			17.6			17.7			17.9						
		Loss Mass	17.6			17.7		19.3		17.5		19.8		20.4							19.8 ± 0.32
Alice	2	3	15.6																		
		6	15.8			14.7	10.4														
		9	16.5			14.3			14.9	14.5	12.7										
		12	16.0			14.0			14.9		14.9	14.9	14.0	15.3							
		15	24.0			13.8		15.4	14.9		14.8	14.9		15.5	14.9	17.0					
		18				13.4			15.0			15.0			15.0						
		Loss Mass	17.6			14.0		15.4		14.9		14.1		15.4						15.0 ± 0.43	
S184	1	3	18.7																		
		6	18.5			18.4	18.7														
		9	18.5			18.5			18.5	18.3											
		12	18.5			18.5			18.4			18.5	18.5								
		15	18.3			18.6		17.5	18.4		26.0	18.5			18.5	17.9					
		18	18.3			18.6			18.4			18.4			18.3						
		Loss Mass	18.5			18.5		17.5		18.4		26.0		18.4						21.8 ± 4.25	
S184	2	3	13.0																		
		6	13.0			15.0	14.0														
		9	13.0			14.9			14.9	14.6											
		12	16.0			14.8			14.7			14.6	14.8								
		15	12.0			14.8		15.7	14.7		13.5	14.7			14.8	14.9					
		18	10.0			14.7			14.8			14.7			14.8						
		Loss Mass	12.8			14.8		15.7		14.8		13.5		14.7						14.6 ± 1.08	

No entries reflect zero or near-zero values in either Table 3 or 4, from which these C:N ratios were derived.