

1 Running title: Soil nitrate control in a Mediterranean vineyard

2 Managing soil nitrate with cover crops and buffer strips in

3 Sicilian vineyards

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14

15 **Abstract**

16 When soil nitrate levels are low, plants suffer nitrogen (N) deficiency but when the

17 levels are excessive, soil nitrates can pollute surface and subsurface waters. Strategies

18 to reduce the nitrate pollution are necessary to reach a sustainable use of resources

19 such as soil, water and plant. Buffer strips and cover crops can contribute to the

20 management of soil nitrates, but little is known of their effectiveness in semiarid

21 vineyards plantations. The research was carried out in the south coast of Sicily (Italy)

22 to evaluate nitrate trend in a vineyard managed both conventionally and using two

23 different cover crops (*Triticum durum* and *Vicia sativa* cover crop). A 10m wide

24 buffer strip was seeded with *Lolium perenne* at the bottom of the vineyard. . Soil

25 nitrate was measured monthly and nitrate movement was monitored by application of
26 a ^{15}N tracer to a narrow strip between the bottom of vineyard and the buffer and non-
27 buffer strips. *L. perenne* biomass yield in the buffer strips and its isotopic nitrogen
28 content were monitored. *V. sativa* cover crop management contribute with an excess
29 of nitrogen, and the soil management determined the nitrogen content at the buffer
30 areas. A 6 m buffer strip reduce the nitrate by 42% with and by 46% with a 9 m buffer
31 strip. Thanks to catch crop, farmers can manage the N content and its distribution into
32 the soil over the year, can reduce fertilizer wastage and reduce N pollution of surface
33 and ground water.

34 Keywords: vineyard; cover crop; ^{15}N ; buffer strip, Mediterranean

35 **1 Introduction**

36 Mediterranean agriculture soils are being subjected to intense land degradation (Cerdà
37 et al., 2010) due to the intensification of the agriculture practices. This intensification
38 increases the misuse of herbicides and pesticides, leading to soil biological
39 degradation (Garcia Orenes et al., 2009) and soil erosion (Cerdà et al., 2009a). The
40 impact of new agriculture systems: highly mechanized, chemical farming and irrigated
41 (see Cerdà et al., 2009b) contributed to environmental problems such as soil and water
42 pollution (Semaan et al., 2007). Last concept of agriculture intensification, applied by
43 new generation of farmers, involves new strategies to avoid those environmental
44 problems, through human labour increase. An example is the use of catch crops to
45 maintain the soil fertility and preserve soil erosion (Novara et al., 2011), as well as the
46 use of geotextiles to reduce the soil losses (Giménez Morera et al., 2011).

47 Over the last decades in the semiarid Mediterranean environment, high external inputs
48 of nitrate and irrigation in intensively managed agricultural systems reduced surface

49 and subsurface water quality (Butturini et al., 2003; Lassaletta et al., 2009). In
50 particular, vineyard soils are conventionally managed and frequently tilled, which
51 reduces the vegetation cover and increases the proportion of bared soil. This induces a
52 high rate of organic matter mineralization and nitrate leaching (Cerejeira et al., 2000).
53 There are other crops in the Mediterranean that are affected by similar problems such
54 as the olive plantations (Gomez et al., 2009) under rainfed production but also on drip
55 irrigated land. Cerdà et al. (2009, b) found an increase of soil erosion rates and soil
56 degradation. However, vineyards are moving fast to drip irrigation and chemical
57 fertilization, little research has been conducted to determine the environmental impacts
58 of the land management change.

59 To reduce the loss of nitrate in soils and the pollution of ground and surface water,
60 European directives have favored Good Agricultural Practices, such as the reduction
61 of mineral nitrogen fertilization or the establishment of vegetated buffer strips
62 (Council Directive 91/676/EEC, 1991). Buffer strips are vegetated zone adjacent to
63 agroforestry or crop fields that intercept and “treat” the water draining the cropland
64 (Dillaha et al., 1988; Dosskey, 2001). Buffer strips reduce the movement of sediment,
65 nutrients, and pesticides from agricultural lands into the ecosystem (Borin et al., 2010;
66 Borin et al., 2002; Patty et al., 1997; Popov et al., 2005; Rankins et al., 2001; Schmitt
67 et al., 1999; Tingle et al., 1998). Buffer effectiveness depends on buffer characteristics
68 such as surface hydraulic properties, vegetation species, soil type, slope morphology,
69 and buffer width (Balestrini et al., 2011; Bharati et al., 2002; Dunn et al., 2011,
70 Schmitt et al., 1999). Buffer strip efficacy is also affected by the agricultural system
71 (land management and crop) and the management practices used in the buffered area
72 (Bedard-Haughn et al., 2005).

73 Although cover cropping reduce nitrate leaching (Ritter et al., 1998; Thomsen, 2005;
74 Tonitto et al., 2006), decreasing soil erosion, (Novara et al., 2011; Quinton and Catt,
75 2004) and improving soil aggregation, water infiltration, and water-holding capacity
76 (Kuo et al., 1997; Villamil et al., 2006), leguminous cover crops can result in excess
77 nitrate content in soil due to his capacity to fix nitrogen.

78 For vineyards, little information is available on how leguminous versus cereal cover
79 crops affect the distribution and retention of soil nitrate during the year. That
80 information is needed to avoid an excess or deficit in soil nitrate according to
81 grapevine needs. Knowledge to avoid excess or deficit of soil nitrate could be useful
82 local policymakers to match different needs between the “Nitrate directive” which
83 limits nitrate use to reduce water pollution and “Agroecological measures” that favour
84 the use of leguminous cover crop to improve farm sustainability.

85 The goals of this study are to: (i) evaluate the spatial and temporal variation in soil
86 nitrate content in a vineyard; (ii) compare the effects of alternative and conventional
87 soil management on soil nitrate content; (iii) compare the effect of leguminous vs.
88 cereal cover crops on soil nitrate dynamics over time; and (iv) evaluate the ability of
89 buffer strips of different widths to retain nitrate.

90

91 **2 Materials and methods**

92 The separated and combined effect of cover crop and buffer strip on nitrate dynamics
93 in vineyard was evaluate in Agrigento province (37° 35' 12''N–13°01' 41'' E; 85 m
94 a.s.l), Sicily (Italy) (Fig. 1). Soils around the study area (Fig. 1) are at high risk of
95 nitrate contamination according to a “map of nitrate vulnerable zone in Sicily” (scale
96 1:250.000) developed by the Sicilian regional government (Regione Sicilia, Decreto

97 D.D.G. n. 121, 2005). Farmers in this area apply conventional soil management (3–4
98 ploughings per year, 0.15 m deep, to bury weeds and aerate the topsoil) which
99 increases the levels of soil nitrate in the groundwater. The nitrate content of the
100 groundwater exceeds 50 mg L⁻¹, which is considered polluted (Nitrates Directive,
101 1991). During the period of observation (March 2006 to May 2009), the ground water
102 nitrate concentration always exceeded 130 mg L⁻¹, with a maximum of 190 mg L⁻¹ in
103 summer (Fig. 1).

104 The study was conducted in a vineyard that lies on a 260° N slope with 7% angle slope.
105 The area has a typical Mediterranean climate with dry, hot summers and moist winters.
106 Precipitation data from Menfi weather station were used. The mean annual precipitation
107 is 516 mm. Most rain falls in autumn and winter, and rainfall is highest in October
108 (monthly mean rainfall of 81 mm) and lowest in July (monthly mean rainfall of 2 mm).
109 On average, 3% of the mean annual rainfall occurs during summer (June, July, and
110 August) while 42% occurs during winter (November, December, and January). The
111 mean annual temperature is 18°C; the hottest months are July and August (monthly
112 means of about 25°C), and the coldest months are January and February (monthly
113 means of 11°C). Soil in the experimental field is classified as a Hapli-Eutric Vertisol
114 according to the World Reference Base for Soil Resources (WRB, 2006).The top soil (0-
115 20 cm) is composed mainly by 57.1% of clay, 34% of silt and 8.9% of sand, measured
116 based on the pipette method (Day, 1965).

117 The research was carried out in a non-irrigated 10-year old vineyard of the Merlot
118 variety. Vine plant density was 4500 plant ha⁻¹, rows were 80 m long with 2.2 m
119 between rows. The experiment included three treatments in which the soil between the
120 vine rows (along slope) was managed with *Triticum durum* L. cover crop (T), with a

121 *Vicia sativa* L. cover crop (V), and by conventional tillage (C) in a randomized block
122 design. The first block or replicate of the experiment is represented in figure 2. Within
123 each block, each treatment was applied to six adjacent inter-rows, giving 18 adjacent
124 inter-row per block. Cover crops were seeded in October 2006 and in October 2007
125 with specialized sod-seeding equipment, and cover crop biomass was turned into the
126 soil by rotary tillage in April 2007 and April 2008. For treatment C, the soil was
127 ploughed 3-4 times per year (0.15m deep, starting after the first rain in September)
128 depending on weeds control needs and to aerate the topsoil. The vineyard was not
129 fertilized.

130 At the bottom of the vineyard and perpendicular to the direction of slope in the field, a
131 strip of soil that was 10 m wide and 80 m long was divided into buffer strips and non-
132 buffer strips so that each treatment plot had one buffer strip and one non-buffer strip at
133 its downslope base (see Fig. 2). The buffer strips were seeded with *Lolium perenne* (40
134 kg of seed per ha⁻¹) in October 2005, one year before the beginning of experiment. The
135 non-buffer strips were managed by conventional tillage. The experiment had a total of
136 three blocks. Soil samples were collected once per month from January 2007 to June
137 2008 from the central inter-row of each treatment. Soil samples (three subsamples for
138 each treatment) were taken at 0-20 cm depth along the slope at intervals of 20 m in the
139 vineyard plot (average of three soil samples for each sampling position) and at
140 intervals of 3 m in the buffer zone (average of three soil samples for each sampling
141 position); the samples within each plot were kept separate so that nitrate content along
142 the slope could be quantified. The positions with respect to slope for both the
143 treatment and strip plots are referred to as upper, middle, and lower. Aboveground
144 biomass of *L. perenne* in the buffer strips was collected in April, May, and June of

145 2007 and 2008 for determination of yield and ^{15}N content. *V. sativa* and *T. durum*
146 biomass were sampled in April of both years (before the cover crops were
147 incorporated into the soil) by removing the aboveground biomass in three 1-m^2 areas
148 per replicate; dry weight and N content were determined.

149 The dynamics of Nitrate in the soil and vegetation of the buffer strip were monitored
150 with nitrogen isotopes, which are stable and nonradioactive (Powlson & Barraclough,
151 1993). We used an ^{15}N -enriched tracer, and the natural abundance background levels
152 of ^{15}N was measured before the application. The tracer was sprayed onto the surface of
153 a 1-m strip of soil that separated the buffer and non-buffer strips from the rows treated
154 with T, V, and C (Fig. 2) in the first week of February for both years (2007 and 2008).
155 The tracer was an aqueous solution of ammonium sulphate (1.57% ^{15}N atom) sprayed
156 at 80 kg ha^{-1} .

157 To monitor water and sediment yield, a 1 m wide Gerlach (Gerlach, 1967; Morgan,
158 1977) with a 40 L deposit was installed at the bottom buffer and non-buffer site.
159 During the study period no water runoff was recorded at the bottom of the plot in both
160 treatments. The cover of *T. durum* and *V. sativa* and the tillage management enhanced
161 the infiltration and, as a consequence, overland flow was negligible.

162 The $\text{NO}_3\text{-N}$ content of the soil samples was determined by aqueous extraction with a
163 Dionex D120 ion chromatograph. Soil and plant samples were subjected to isotopic
164 analysis with an EA-IRMS (elemental analyser-isotopic ratio mass spectrometer). An
165 automatic sampler was used, and the samples were combusted in the presence of
166 oxygen at $1050\text{ }^\circ\text{C}$.

167 Isotopic levels for the soils and plants are reported as atom % ^{15}N excess (IE), which
168 refers to the amount of ^{15}N present relative to the average naturally occurring

169 background ^{15}N levels occurring in the biomass and the soil under the specific
170 experimental conditions. Background levels are based on pre-application samples.
171 Where possible, atom % ^{15}N excess amounts were extrapolated to get the total amount of
172 ^{15}N in a given pool by weight and thus to determine a ^{15}N budget (Bedard-Haughn and
173 van Kessel, 2004).

174 The international standard for N is atmospheric nitrogen (Mariotti, 1983 and 1984), for
175 which the $^{15}\text{N}/^{14}\text{N}$ ratio is 0.003676. The international reference materials IAEA-N1 (δ
176 $^{15}\text{N} = 0.03\text{‰}$), IAEA-N2 ($\delta^{15}\text{N} = 20.1\text{‰}$) and IAEA-N3 ($\delta^{15}\text{N} = 4.5\text{‰}$) were used for
177 calibration and normalization following the study of Bohlke et al. (2003). Analytical
178 precision is about 0.2‰. A split plot design with three replications was used in which
179 management was the main plot and elevations (position on slope, i.e., upper, middle,
180 and lower) were subplots. After testing normal distribution of data, statistical analysis
181 was carried out separately for the managed area (treatments T, V, or C) and the buffer
182 strip/non-buffer strip area on the quantity of ^{15}N detected in buffer strips and non-
183 buffer strips below the treatment plot and nitrate. Nitrate content was also compared
184 using repeated measure ANOVA carried out according the used experimental design
185 (SAS, 2002)

186

187 **3 Results and discussion**

188 **3.1 Soil nitrate content in the vineyard**

189 The nitrate content was 12.4% greater in treatment V vs. C but only 1.71% greater in
190 treatment T vs. C. These significant differences ($P \leq 0.0001$) (Table 1) can be explained
191 by the ability of the legume, *V. sativa*, to fix N and by the high N content of the
192 legume tissue. Before the cover crops were incorporated into the soil, the aboveground

193 dry biomass was $11 \pm 1.2 \text{ Mg ha}^{-1}$ (with 2.8% N content) for treatment V and 8.33 ± 2.1
194 Mg ha^{-1} (with 1.3% N content) for treatment T.

195 Our results are in agreement with previous studies, which observed that cover crops
196 increase soil N content and soil organic matter (SOM) content (Jackson et al, 2004;
197 Sainju et al., 2000, Ramos et al., 2010). At the same way, in the experimental site we
198 hypothesized a SOM increase after moving catch crops into the soil. The potential to
199 immobilize and retain soil N increases with SOM content (Barretta and Burke, 2000).
200 Continuous tillage under conventional management, in contrast, causes bare soil (weed
201 free) and an increased N loss due to leaching, short-term bursts of mineralization of
202 organic N substrates, and nitrous dioxide efflux (Grandy and Robertson, 2006).
203 Consequently, soil nitrate content was lower in the conventionally managed plots than
204 in the plots with catch crops.

205 Soil nitrate content changed during the year, apparently because of precipitation,
206 mineralization, uptake by vines and cover crops. Cover crops have direct and indirect
207 effects on soil fertility and vine nutrition. Incorporation of leguminous catch crops
208 directly adds organic nitrogen to the soil (Nakhone and Tabatabai, 2008). After
209 mineralization, which begins within weeks after incorporation, this nitrogen is
210 available for vine uptake (Rupp, 1996). In contrast, non-leguminous cover crops often
211 result in the depletion of the vineyard nitrogen pool (Celette et al., 2009). The
212 interactions between cover crop, soil fertility, and vine growth are complex and
213 dynamic. Measuring and predicting changes in soil nutrient status can be far more
214 difficult in cover-cropped vineyards than in vineyards managed with chemical
215 fertilizers alone, as the farmer controls the source of nitrogen.

216 Soil nitrate values were lower in spring and high in late summer or autumn (Fig. 3).
217 The values ranged from 1.45 to 26.56 mg L⁻¹ under conventional tillage, from 1.71 to
218 28.14 mg L⁻¹ under the *V. sativa* cover crop, and from 1.87 to 19.71mg L⁻¹ under the
219 *T. durum* cover crop.

220 The strong decrease of nitrate from winter to early spring can be attributed to leaching
221 in response to precipitation under conventional tillage (Davidson, 1992) and to plant
222 uptake of N in the cover crop treatments (Steenwerth and Belina, 2008). In summer,
223 the high increase in nitrate under the *V. sativa* cover crop and with conventional tillage
224 might be explained by rapid mineralization of organic matter. The peak in soil nitrate
225 was lower with the *T. durum* treatment than with the other two treatments, probably
226 because of the relatively high carbon to nitrogen ratio and lignin content of *T. durum*,
227 which would reduce its rate of mineralization when it was incorporated into the soil.

228 Considering the average of nitrate content measured in the first and second year,
229 respectively not significantly difference were found for the conventionally managed
230 plots and for *V. sativa* plots. On the contrary, the values were higher in the second year
231 in the *T. durum* plots. The latter difference is consistent with previous reports that N
232 can be immobilized following the planting of some cereal cover crops (Fageria et al.,
233 2005).

234 In all treatments, soil nitrate content increased down the slope. The soil nitrate content
235 in the middle and lower tram of the slope increased by 48 and 112% in the *V. sativa*
236 plots, by 47 and 123% in the *T. durum* plots, and by 37 and 94% in the conventionally
237 managed plots. This data demonstrate the leaching of nitrates by the surface wash and
238 subsurface wash, and confirm that the bottom slope buffer strips of the slope can be a
239 good strategy to avoid the pollution with nitrates. The results showed that nitrates are

240 leached by surface and subsurface wash. It also confirms that the buffer strips are a
241 good strategy to mitigate nitrate soil pollution”

242

243 **3.2 Soil nitrate in the buffer and non-buffer strips**

244 Soil nitrate content was higher in the non-buffer strips (without *L. perenne*) than in the
245 buffer strips (with *L. perenne*) and was higher in late summer and autumn than in
246 spring (Fig. 3). Soil nitrate content ranged from 2.26 to 12.5 mg L⁻¹ in buffer strips and
247 from 1.5 to 30.5 mg L⁻¹ in the non-buffer strip (Fig. 3). This result is consistent with
248 the idea that a buffer strip can capture excess nitrate during the whole year around and
249 retain nitrate in soil during the rainiest periods and thereby reduce leaching (Leeds-
250 Harrison et al., 1999). Note that large quantity of nitrate that accumulated during
251 summer (a period with low precipitation) in the non-buffer strips was completely lost
252 by the end of winter in both years of observation due to runoff and leaching.

253 Soil Nitrate content was 25% and 35% higher in the middle and lower slope positions
254 than in the upper slope position. In the buffer strips, however, nitrate content was only
255 12% and 14% higher in the middle and lower positions than in the upper position.
256 These results agree with other research concerning N removal as a function of buffer
257 width (Bedard-Haughn et al., 2005; Dillaha et al., 1989). Statistical analysis showed
258 differences only between upper and middle position and between the upper and lowest
259 position of the strips (Table 1).

260 Soil nitrate content in buffer (Table 1) and non-buffer strips was significantly affected
261 by vineyard soil management (treatments V, T, and C). In the non-buffer strip,
262 differences were significant only during summer, when soil nitrate content was highest
263 with treatment V, lowest with treatment C, and intermediate with treatment T. In the

264 buffer strips, soil nitrate over all sampling times tended to be highest with treatment C
265 (8.1 mg L⁻¹), followed by treatment T (6.1 mg L⁻¹), and treatment V (5.7 mg L⁻¹).
266 These differences are consistent with the effects of vineyard soil management on
267 losses of nitrate by subsurface water flow.

268 **3.2 ¹⁵N tracer in biomass**

269 The mean dry biomass of *L. perenne* in the buffer strips was unaffected by soil
270 management treatments and averaged 4.14±0.85 Mg ha⁻¹. The ¹⁵N uptake by *L.*
271 *perenne* tended to decrease with distance from the narrow strip where ¹⁵N was applied,
272 and was significantly greater at 3 m downslope than at 6 or 9 m downslope but did not
273 differ between 6 and 9 m downslope (Fig. 4). The *L. perenne* located 6m and 9m from
274 the application zone contained 72 and 76% less isotopic excess, respectively, than the
275 *L. perenne* located only 3m from the application zone. Values of isotopic excess
276 ranged from 0.01±0.038‰ to 0.0018±0.0007‰ after the first application and from
277 0.0329 ±0.011‰ to 0.0019±0.0006‰ after the second application of ¹⁵N tracer (Fig.
278 4).

279 The ¹⁵N excess decreased over time after each application (Fig. 4), indicating dilution
280 of the ¹⁵N signature caused by uptake of non-enriched N (Bedard-Haughn et al., 2004).

281 **3.3 ¹⁵N tracer in soil**

282 ¹⁵N isotopic excess in the soil was higher in the non-buffer strips than in the buffer
283 strips (Fig. 5). In the buffer strip soil, atom % ¹⁵N excess ranged from
284 0.0028±0.0011‰ to 0.0042±0.0013‰ after the first application, and from
285 0.0026±0.0002‰ to 0.0074±0.0017‰ after the second application of ¹⁵N tracer (Fig.
286 5). Averaged over time and location in the strip, values were 15% higher in the non-
287 buffer strips than in the buffer strips.

288 Relatively to the content of ^{15}N isotopic excess in the buffer strip soil at 3 m from the
289 application site, the content was 42% and 46% lower at 6m and 9m, respectively, from
290 the application zone (Fig. 5). In contrast, the content of ^{15}N isotopic excess in the non-
291 buffer strip increased with distance down the slope from the application site. Relative
292 to the content of ^{15}N isotopic excess in the non-buffer strip soil at 3 m from the
293 application site, the content in the buffer strip was 4 and 91% higher at 6 m and 9m,
294 respectively, from the application zone (Fig. 5).

295

296 **3.4 Implications**

297 Vineyard plantations in the Mediterranean region must be managed with a cover crop
298 and a vegetative buffer strip in order to reduce nitrates excessive concentration in
299 soils and water. Relative to conventional tillage, a cover crop provides a better
300 distribution of soil nitrogen in time and space with respect to grapevine growth
301 requirements, while the vegetative buffer strip captures the excess nitrate and thereby
302 reduces pollution of surface and ground water by nitrate. Soil nitrate content was
303 lower with conventional tillage (108 kg ha^{-1}) than with the cover crops (110 kg ha^{-1} for
304 *T. durum* and 122 kg ha^{-1} for *V. sativa*), with a peak during the hottest period of the
305 year (August). This temporal distribution of soil nitrate does not fit to grapevine
306 nutrients needs and as a consequence there is a nutrient deficit during the growing
307 period and an excess in summer until the first rain in autumn, when nitrate are leached
308 and eroded by the surface wash. Soil under conventional tillage is unable to retain
309 nitrate, and then, N fertilization is applied. This will increase the N wash.

310 In the Mediterranean area, soil management with a cover crop in the winter reduces
311 the soil, water and nitrates losses. This research demonstrates that cover or catch crops

312 contribute to the nitrogen fixation (*V. sativa*). Because of biological fixation of N,
313 leguminous cover crops provide a net N input to vineyards (King and Berry 2005;
314 Drinkwater et al., 1998).

315 Cover crops are a valuable alternative to chemical fertilizers in vineyards, it is
316 important to evaluate not only the amount of N added to the system but also the N
317 availability during the year. In our case, the *V. sativa* cover crop experienced a high
318 mineralization rate in summer, when grapevines cannot utilize nitrate. Because it
319 contributes less nitrogen and has a higher C:N ratio, the *T. durum* cover crop may
320 result in less pollution and greater retention of N than either the *V. sativa* cover crop or
321 the conventional soil management system.

322 The high nitrate content at the edge of vineyard is likely to move into water supplies
323 unless captured by a buffer strip. Buffer effectiveness in reducing nitrate loss was
324 demonstrated by the use of a ¹⁵N tracer. Most of the applied ¹⁵N tracer was found in
325 the biomass of *L. perenne* in the first 3m of the buffer strip, indicating that the ¹⁵N
326 tracer in soil decreased down the slope of the buffer strips. Although nitrate decrease
327 varied with buffer width, we considered a 6m buffer strip sufficient to control nitrate
328 pollution in vineyards, like the most of Sicilian vineyards, with a slope of about 7%.

329

330 **4 Conclusions**

331 Cover crops in vineyard under Mediterranean climatic conditions inter-row reduce
332 water runoff and act as catch crop regulating nitrate availability during the year.
333 Thanks to catch crop, farmers can manage the N content and its distribution into the
334 soil over the year. In this way, vineyard managers can reduce fertilizer wastage and
335 reduce N pollution of surface and ground water. The main contribution of this research

336 is that nitrate losses can be managed in vineyards successfully by the use of
337 appropriate soil conservation practices and by installation of buffer strips.

338

339

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508

509 **Figure caption**

510 Figure 1. Study area (top), monthly rainfall (red bars) and average groundwater nitrate
511 concentration from 2006 to 2008 (green line).

512

513 Figure 2. Experimental design.

514

515 Figure 3. Soil nitrate content in vineyard plot (a) (average of upper, middle and lower
516 position), buffer strips (b) and non-buffer strips (c) below the *V. sativa* cover crop
517 (black line), the *Triticum durum* cover crop (grey line), and conventional tillage
518 (broken line). Horizontal lines represent statistical difference ($p < 0.005$) according to
519 ANOVA repeated measure statistical analysis test.

520

521 Figure 4. Atom % ^{15}N excess in *Lolium perenne* in the buffer strips vs.the time. Black,
522 grey, and white bars represent 3, 6, and 9m distances (3 samples for each distance),
523 respectively, from the ^{15}N application zone. The vertical lines represent standard
524 deviation.

525

526 Figure 5. Atom % ^{15}N excess in soil over the time in the buffer strips and non-buffer
527 strips. Black, grey, and white bars represent 3, 6, and 9m distances (3 samples for each

528 distance), respectively, from the ^{15}N application zone. The vertical lines represent
529 standard deviation.

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531

532 **Table Caption**

533 Table 1. Results of the Anova test for NO₃-N in vineyard and buffer zone

534

		Vineyard	Buffer	No buffer
535				
		DF		
	Soil Management (M)	2	<0.0001	<0.0001
536	Slope position (S)	2	0.0307	0.0405
	M x S	4	0.9221	0.7687
537	Time (T)	17	<0.0001	<0.0001
	M x T	34	<0.0001	0.2601

538

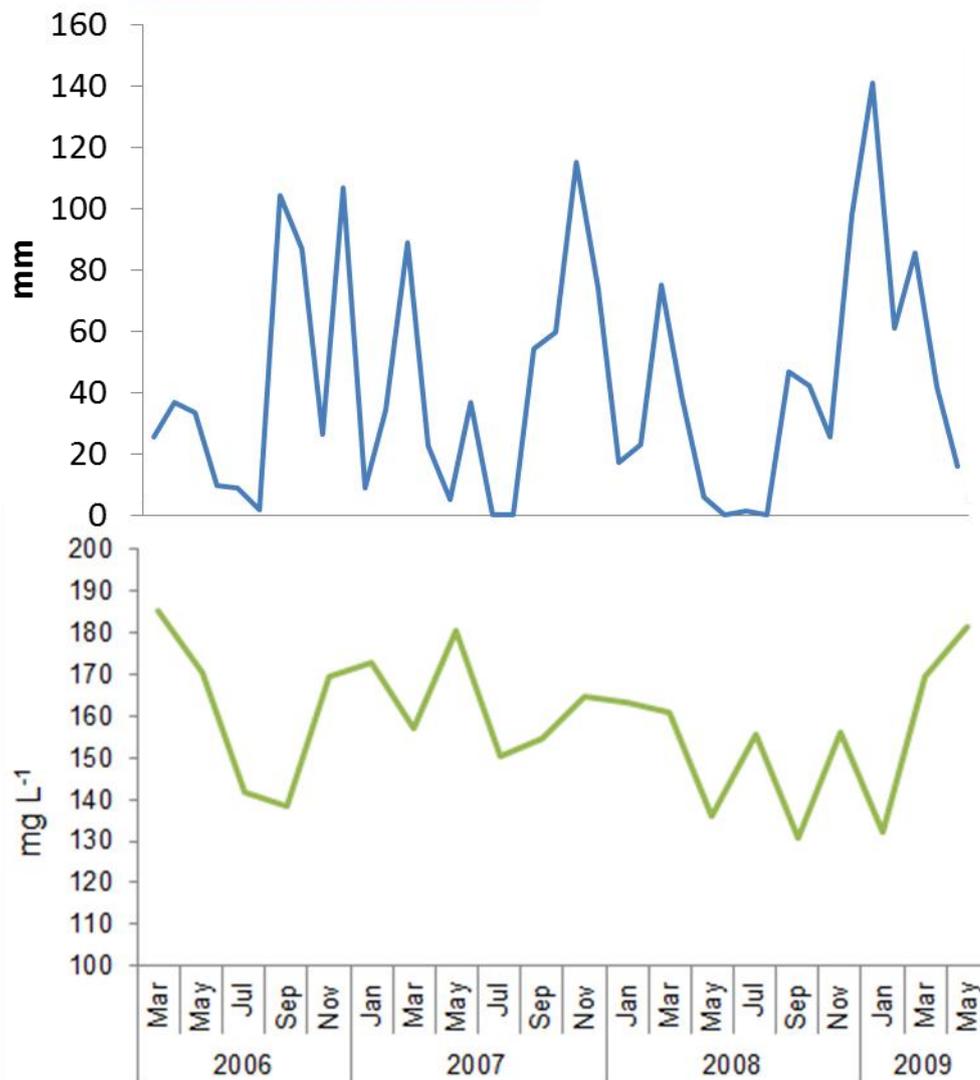
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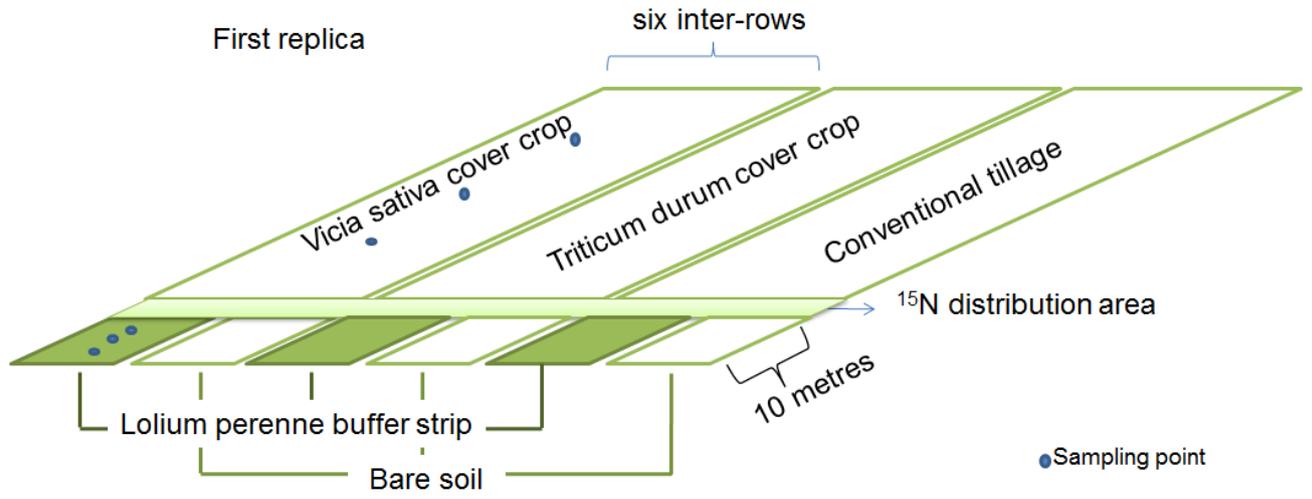
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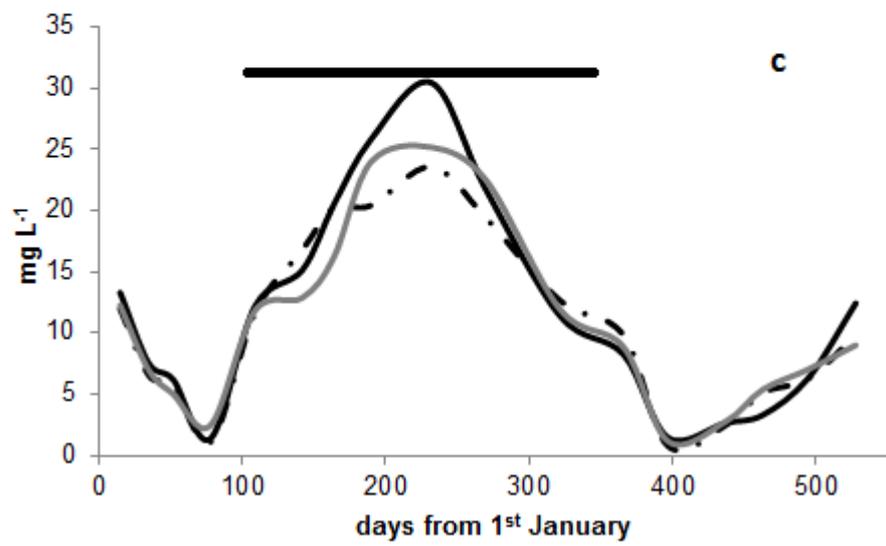
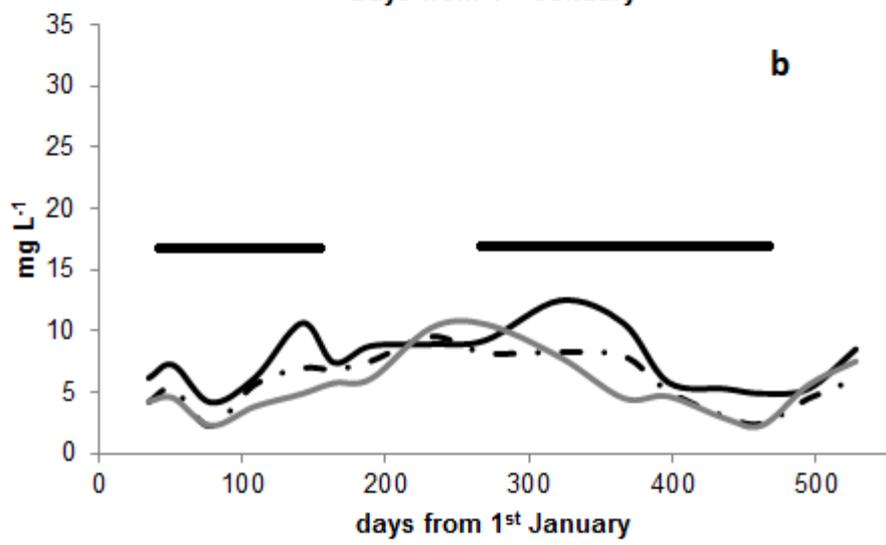
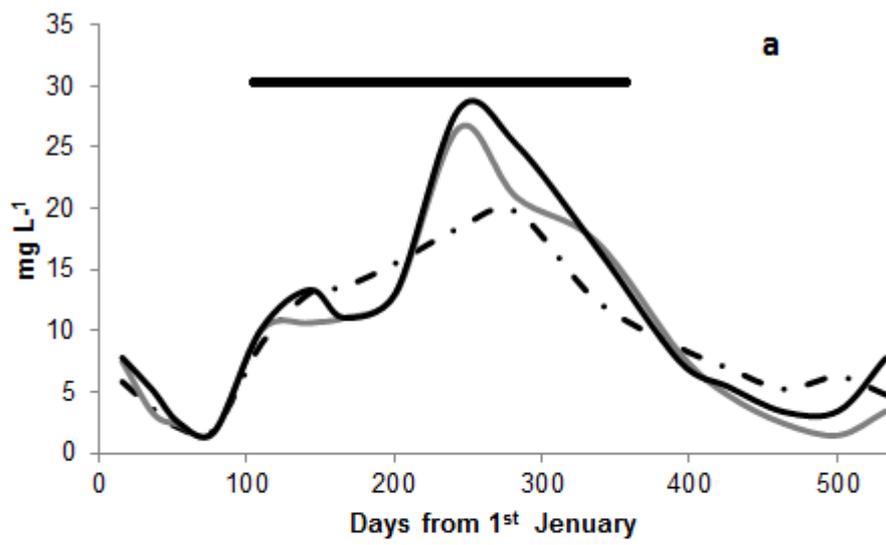
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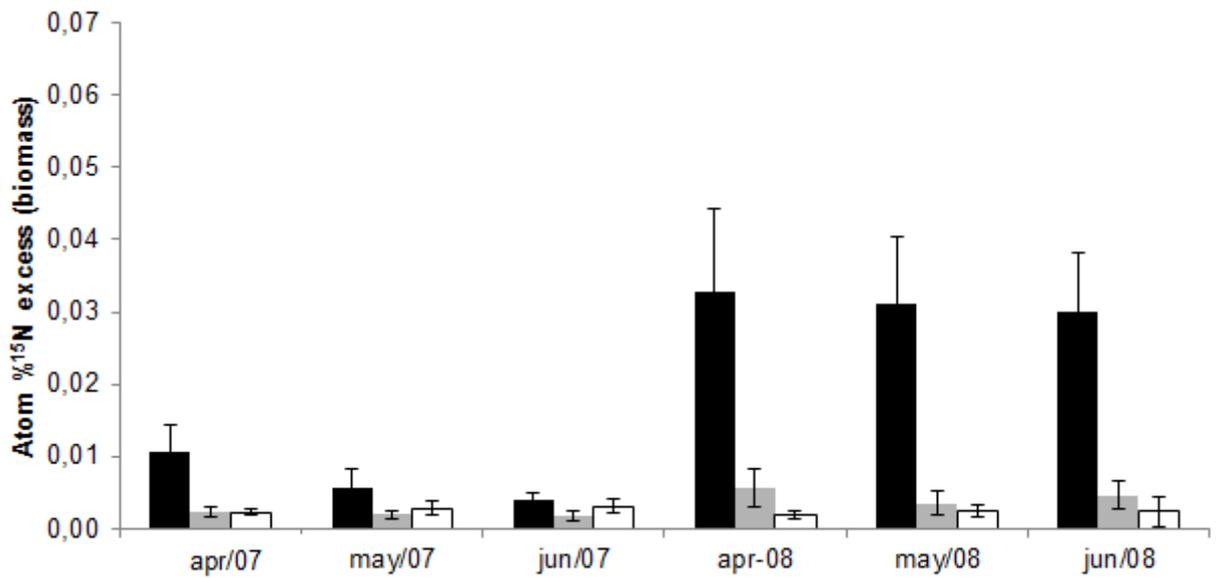
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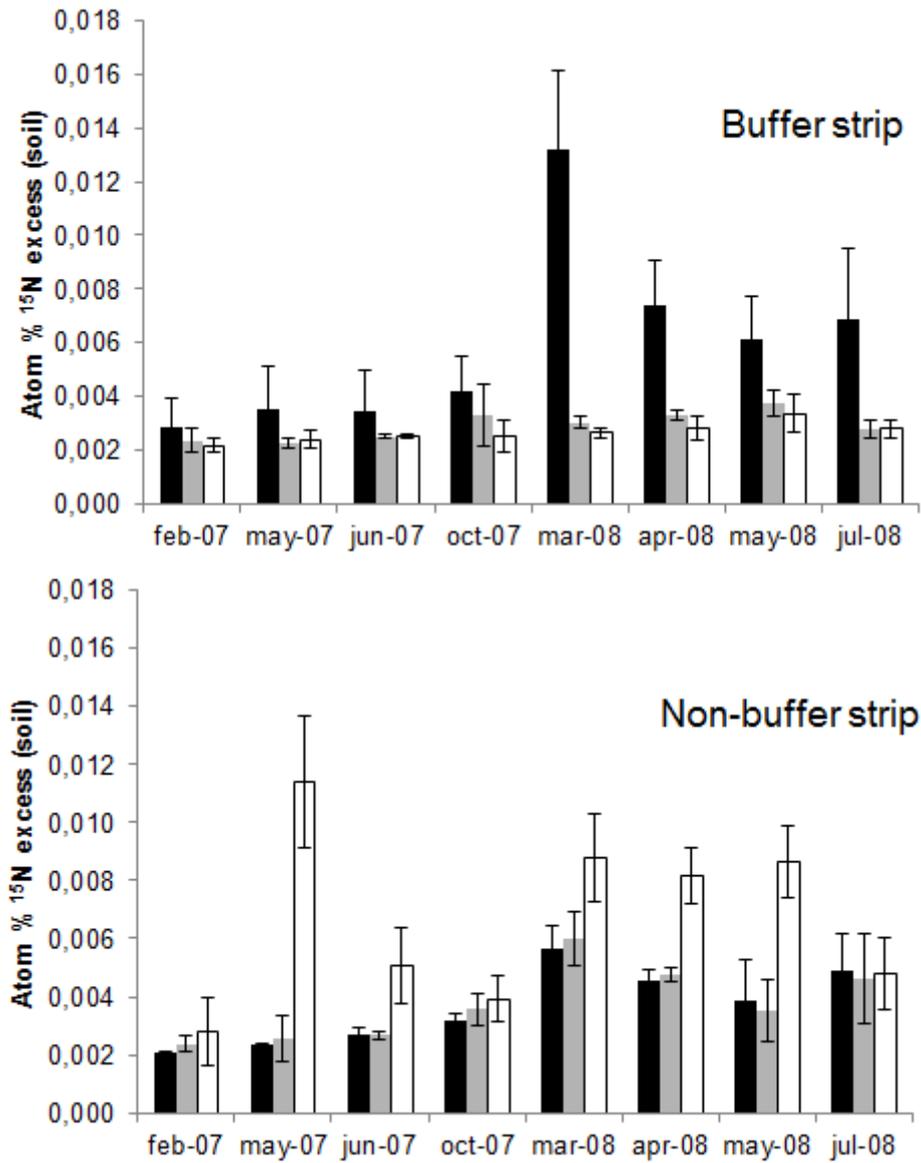
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