

1 **Online Supplementary Materials**

2 **Nitrogen fluxes at the root-soil interface show a mismatch of nitrogen fertilizer supply and root uptake capacity**

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7 **Table S1.** Ammonium-N, nitrate-N and amino (AA)-N fluxes and concentrations in the three soils under sugarcane. Numbers in parentheses are  
 8 standard error of the mean of 40-48 replicate soil extractions or flux measurements. Capital superscript letters represent significance ( $P < 0.05$ )  
 9 from a Tukey's post-hoc test comparing soil types within each N form and type of N sampling.

	Diffusive flux (nmol N cm <sup>-2</sup> h <sup>-1</sup> )			Free N (μmol N kg <sup>-1</sup> dry soil)			Exchangeable N (μmol N kg <sup>-1</sup> dry soil)		
	Urea fertilised	Organic fertilised	Unfertilised	Urea fertilised	Organic fertilised	Unfertilised	Urea fertilised	Organic fertilised	Unfertilised
<b>NH<sub>4</sub><sup>+</sup></b>	3017 (515) <sup>A</sup>	28 (2) <sup>B</sup>	4 (0.3) <sup>C</sup>	9877 (1384) <sup>A</sup>	366 (27) <sup>B</sup>	5 (1) <sup>C</sup>	14246 (1642) <sup>A</sup>	1534 (140) <sup>B</sup>	50 (6) <sup>C</sup>
<b>NO<sub>3</sub><sup>-</sup></b>	27 (14) <sup>B</sup>	68 (4) <sup>A</sup>	1 (0.2) <sup>C</sup>	378 (55) <sup>A</sup>	450 (29) <sup>A</sup>	13 (1) <sup>B</sup>	1005 (533) <sup>A</sup>	407 (27) <sup>A</sup>	40 (5) <sup>B</sup>
<b>AA</b>	13 (1) <sup>B</sup>	19 (1.4) <sup>A</sup>	14 (1.2) <sup>B</sup>	4 (1) <sup>A</sup>	2 (0.3) <sup>A</sup>	5 (0.4) <sup>A</sup>	51 (3) <sup>A</sup>	50 (2) <sup>A</sup>	48 (2) <sup>A</sup>
<b>Total</b>	<b>3057</b>	<b>115</b>	<b>19</b>	<b>10259</b>	<b>818</b>	<b>23</b>	<b>15302</b>	<b>1991</b>	<b>138</b>
<b>LMW-N</b>									

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11 **Table S2.** Soil properties of the studied commercial fields under sugarcane. Data represents  
 12 averages of five replicate soil samples, with standard error of the mean in parentheses.

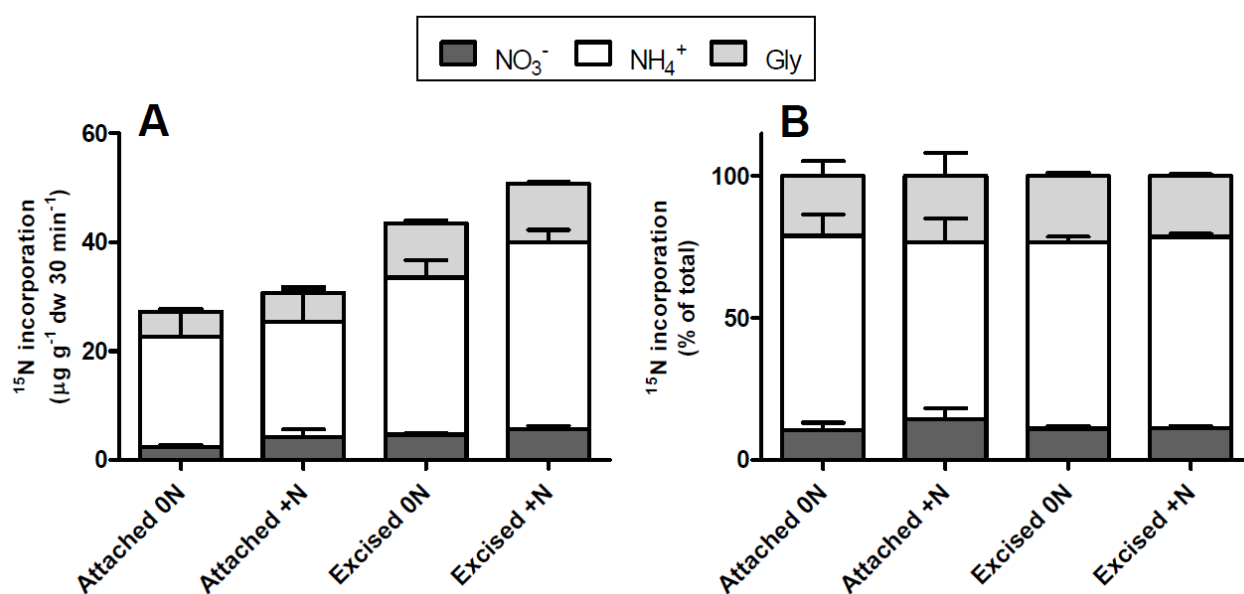
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**Soil and climate**

Soil type	Hydrosol (Australian Soils Classification)
pH (H <sub>2</sub> O)	4.87 (0.1)
Electrical conductivity (μS cm <sup>-1</sup> )	315 (59.6)
Organic carbon (%C)	2.1 (0.1)
Total nitrogen (%N)	0.28 (0.003)
Annual mean maximum temperature (°C)	25.1
Annual mean minimum temperature (°C)	17.2
Annual rainfall (mm)	1300

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17 **Figure S1.** Comparing the uptake of nitrate, glycine and ammonium by attached and excised  
 18 roots incubated in equimolar solution of the three N sources with one N source <sup>15</sup>N-labelled  
 19 and two N sources unlabelled (1000 μM each N source). Roots were excavated from fertilised  
 20 (+N) or unfertilised (ON) sugarcane plants in a field trial near Bundaberg, Queensland. The  
 21 uptake experiment was conducted in summer (January).

22 **Supplementary methods:**

23 **Nitrogen source selectivity of attached roots**

24 Experiments with attached roots were carried out to validate or reject results from the excised  
25 root experiments at one time point and with one N concentration. The experiment was  
26 conducted in summer (23 and 24 January) and the air temperature during the experiment was  
27 30–35°C. Roots, including tips, were excavated by carefully brushing away soil from the soil  
28 surface until roots were exposed. The roots used in the *in situ* experiment were young, fleshy,  
29 white roots including the tip and elongation zone of the major root axis, as well as branch  
30 roots above this region similar to the roots used in the excised roots experiments. Roots were  
31 excavated by hand from approximately 0–10 cm depth. Roots were left attached to plants,  
32 covered with damp paper towel to keep moist and incubated within 3 h of excavation. For  
33 each plant, roots were divided into three groups. Each set of roots was placed in a vial  
34 containing a 3000 µM N solution with equimolar concentrations of each of ammonium (as  
35 (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>), nitrate (as KNO<sub>3</sub>) and glycine (1000 µM N each source), with one of the N  
36 sources labelled with <sup>15</sup>N (98–99 atom%) and the other two N sources at natural abundance  
37 level. Roots were incubated for 30 min before being severed just above the vial, rinsed for 10  
38 min with 10 mM KCl, followed by a 1 min rinse with rainwater. Root samples were dried and  
39 analysed as described above for excised roots.

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41 **<sup>15</sup>N uptake by attached roots**

42 Attached roots from fertilised and unfertilised plants acquired similar amounts of the three  
43 offered N sources (Fig. 3.6). Attached roots incorporated on average 38% less <sup>15</sup>N from all N  
44 sources than excised roots (p<0.05; Fig. 3.5), but proportions of <sup>15</sup>N incorporation of the  
45 three N sources were similar in attached and excised roots regardless of fertiliser treatment.  
46 This suggests the possibility that excised root experiments over-estimate N uptake rates: if  
47 this is the case, then I<sub>max</sub> values would be smaller than those reported here by ~30%. The potentially  
48 higher I<sub>max</sub> values from excised roots which we have used represent a conservative estimate of the  
49 mismatch between soil fluxes and root uptake.

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