Amino Acids: A short-term nitrogen source for establishing irrigated pastures

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Abstract
Although rainfed pasture is the main source of feed for dairy, meat and wool-producing livestock, irrigation is transforming regions by providing moisture, thereby extending growing seasons. Consequently, irrigated pastures require increased fertiliser application notably nitrogen (N) to sustain growth. During establishment grasses actively seek N via roots. However, applications of synthetic N fertiliser may not be fully utilised by immature roots resulting in nitrate leaching. Under irrigation, nutrient leaching poses a risk to the environment. Alternative N from amino acids (AA) transferred by clover roots sown with grasses may reduce additional N inputs until plants are established. To investigate root interactions perennial ryegrass (*Lolium perenne*; PRG) cv. Reward, was sown in pots with either stoloniferous red clover (*Trifolium pratense*; SRC) cv. Rubitas, white clover (*Trifolium repens*; WC) cv. Bounty or Talish clover (*Trifolium tumens*; TT) cv. Permatas creating a 30:70 or 70:30 grass clover composition or monocultures of 30:0 and 70:0 grass. Dry matter (DM) yields of PRG were not significantly ($P > 0.05$) different when clover was present at 30% and 70% compared with PRG alone. When comparing PRG 30:70 sown with either SRC, WC and TT and PRG sown alone at 30:00, the total AA (mg/g DW) of extracted PGR from roots was significantly ($P<0.05$) higher. Treatments analysed by species show the same significant result for PRG 30:70. Results suggest sowing PRG with clover at 30:70 may provide short-term N supply at establishment.

Keywords
Ammonia, exudates, legumes.

Introduction
Red clover (*Trifolium pratense* L.; RC) is a valuable forage species exhibiting yield and animal dietary attributes, along with benefits to the soil environment via N fixation capability (Taylor and Quesenberry 1996). One disadvantage commonly associated with RC is longevity, with yields declining after 2-3 years. Stoloniferous red clover (*T. pratense var. stoloniferum*; SRC) cv Rubitas has the same characteristics as red clover with an adaptation that allows stolon's to vegetatively reproduce plantlets similar to SRC cv. Astred (Hyslop et al. 1996). These vigorous plantlets effectively negate the longevity problem as new plants regenerate the sward. Although most research effort has focused on the persistence of SRC, little work has explored its role during pasture establishment, particularly under irrigation.

Sowing legumes with grasses improves long-term N supply to pasture and the dietary protein intake of grazing animals. However, clover is rarely considered a useful companion during pasture establishment. Sowing grass swards that are very responsive to synthetic N under irrigation and the practice of applying N is now common. The environmental risk under irrigation is nitrate leaching as establishing plant roots only exploit a small fraction of the soil profile. A potential mechanism for short-term N transfer involves exudation of N compounds into the soil by one plant and the absorption of these compounds by another plant. Amino acids, organic acids, simple sugars, polysaccharides and proteins make up plant root exudates. Though root exudation of N in mature clover plants is known to occur, exudation of N in establishing clover plants is complicated by volatility of storage enzyme reactions (Glevarec et al. 2004). To address this complication a pot and lysimeter experiment (Paynel et al. 2001) sown with PRG, WC and RC concluded there was N transfer as AA via root exudates. Amino Acids transported to maturing leaf -tissue in the xylem, then move away from the leaf via the phloem to other leaves or roots (Ortiz-Lopez et al. 2000). This experiment aimed to investigate the amounts of amino acids in the roots at establishment, as an indicative measure of N activity in grass and transfer from companion clovers.

Methods

A pot experiment was established 27th December 2015 on a weedmat within a double fenced enclosure on Flinders Island (40° 6' 8" S 148° 0'14" E) Tasmania. The potting mix was a custom blend of pine bark, washed sand and peat moss at a ratio of 8-2-0.5 with 7 kg fine dolomite lime (200µm), 1.5 kg Limil Lime and 2 kg slow release Basacote™ Plus K-6 month, 11:3:9:15:8 which includes trace elements. White 150 mm diameter pots were thoroughly washed with a 20 % solution of Domestos™ bleach, rinsed and dried prior to filling with potting mix. The experiment was a completely randomised design consisting of 4 species with 8 treatments of 4 pots replicated 4 times. Species sown and treatments were (*Lolium perenne*; PRG) cv. Reward were either 7 or 3 plants per pot; from here referred to as PGR 70 and PGR 30 respectively and mixed sward combinations of either 70:30 or 30:70 grass clover. The clovers sown with PRG were either stoloniferous red clover (*Trifolium pratense*; SRC) cv. Rubitas, white clover (*Trifolium repens*; WC) cv. Bounty or Talish clover (*Trifolium tumens*; TT) cv. Permatas all scarified and inoculated with Rhizobium Group B as described in (Farmnotes 1985). Irrigation was applied at 20 mm equivalent rate when no rainfall was recorded, or when weighed pots were below 85% field capacity by volume.

At 35 days after sowing (DAS), plants were harvested leaving a 50 mm residual and the material weighed and dried at 56 °C for 48 hours. At 50 DAS (D1) and again at 57 DAS (D2) 4 pots from each treatment were destructively harvested. Roots were removed sorted by species and nodules present on clovers counted and recorded. All above and below ground plant material was weighed and dried at 56° C for 48 hours. All grass roots from (D2) were sent for AA analysis at the Australian proteome analysis facility (APAF). Methionine was detected in all samples but was not quantified because of greater than acceptable variation between replicates recorded for this amino acid only. All data sets were analysed assuming a completely randomised design using Proc Mixed software of the SAS System version 9.3 [SAS Institute Inc. 2014. SAS/STAT ® 13.2 User's Guide. Cary, NC: SAS Institute Inc.]. Effects were considered significant at $P<0.05$.

Results

Dry matter yield

At 35 DAS the grass component of the PRG 30:70 grass-clover sward showed a higher DM yield than PRG 30 but only in combination with SRC and WC. The 70:30 grass clover swards were similar in DM yield of the grass only sward (Figure 1A). In contrast to the harvest conducted at 35 DAS, the destructive harvest at 57 DAS showed that the PRG alone treatments yielded slightly higher when the grass component was compared with combinations that included SRC, WC and TT (Figure 1B).

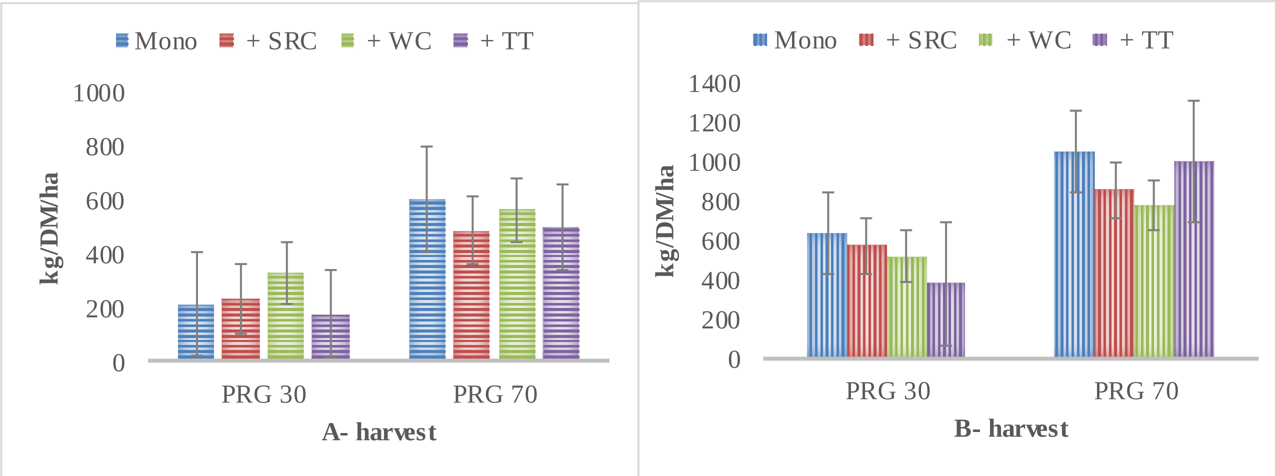

Figure 1. The mean dry matter yield (kg/DM/ha) for the grass only component for treatments sown as either monocultures (Mono) of PRG at 30% or 70% or combinations with stoloniferous red clover (SRC), white clover (WC) or Talish clover (TT), 1A- all harvested to 50 mm residuals 35 days after sowing.1B all destructively harvested to ground level at 57 days after sowing. Standard errors of means shown as error bars.

The grass DM yield was higher in the 70:30 grass clover swards than the 30:70 grass clover swards. The DM yield for the clover component (data not shown) found the 70% grass treatment significantly ($P<0.05$) reduced the clover DM yield in combination with WC and TT. The SRC treatment had no difference in DM yield when sown with 70% or 30% PRG.

Amino Acid results

The total AA concentration (mg/g) of the PGR roots (Table 1) were higher when sown at a grass clover rate of 30:70 than when sown alone. In contrast, when sown at a grass clover rate of 70:30, only inclusion of TT resulted in a higher PGR root AA concentration than PGR sown alone. Arginine, Glycine, Lysine, Phenylalanine and Serine concentration in PGR roots were significantly different between monoculture PRG and treatments sown with clover (Tables 2 and 3).

Table 1. The means and standard error of means for total amino acids concentration (mg/g dry weight) of PRG roots taken from four pots of eight grass or grass clover treatments 57 days after sowing. *Calculation based on amino acid residue mass in protein (molecular weight minus H2O).

	Target % grass	Amino Acid * (mg/g)
Reward PRG Mono	30	31.67 ± 3.47
	70	34.98 ± 4.40
Reward/SRC Rubitas	30	36.51 ± 3.86
	70	32.47 ± 3.40
Reward/WC Bounty	30	41.67 ± 6.60
	70	33.66 ± 3.90
Reward/TT Permatas	30	40.82 ± 4.05
	70	37.49 ± 4.40

Table 2. Predicted means for concentrations of individual amino acids (mg/g dry weight) in perennial ryegrass PRG roots for treatments sown without (w/o) or with (w/c) clover. LS means with the same letter are not significantly different after adjustment using Tukey's method.

species	Arginine	Glycine	Histidine	Lysine	Phenylalanine	Serine	Tyrosine
PRG 30 w/o	1.87^{B}	1.82^{B}	ns	2.03^{B}	1.66^{B}	1.81^{B}	ns
PRG 30 w/c	2.94^{A}	2.22^{A}	ns	2.72^{A}	2.09^{A}	2.28^{A}	ns
PRG 70 w/o	2.14^{AB}	2.00^{AB}	ns	2.32^{AB}	1.82^{AB}	1.97^{AB}	ns
PRG 70 w/c	2.12^{AB}	1.82^{B}	ns	2.32^{AB}	1.84^{AB}	1.99^{AB}	ns

Table 3. Mean individual amino acid concentrations (mg/g dry weight) in perennial ryegrass (PRG) roots for treatments sown as either PRG monocultures or PRG in combination with stoloniferous red clover (SRC), white clover (WC) and Talish clover (TT) at 30:70 or 70:30 grass-clover composition. LS means with the same letter are not significantly different after adjustment using Tukey's method.

species	Arginine	Glycine	Histidine	Lysine	Phenylalanine	Serine	Tyrosine
PRG 30	2.64^{A}	ns	1.05^{B}	3.20^{B}	2.42^{A}	3.62^{A}	1.08^{ABC}
PRG 70	2.89^{A}	ns	1.07^{A}	3.48^{AB}	2.53^{A}	3.72^{A}	1.23^{ABC}
SRC 30	2.90^{A}	ns	1.47^{A}	3.49^{AB}	2.59^{A}	3.78^{A}	0.98^{BC}
SRC 70	2.74^{A}	ns	1.39^{A}	3.35^{AB}	2.44^{A}	3.70^{A}	0.89^{C}
WC 30	3.18^{A}	ns	1.28^{A}	3.92^{AB}	2.84^{A}	4.07^{A}	1.38^{A}
WC 70	2.83^{A}	ns	1.26^{A}	3.44^{AB}	2.55^{A}	3.76^{A}	1.04^{ABC}
TT 30	3.08^{A}	ns	1.30^{A}	4.03^{A}	2.79^{A}	4.15^{A}	1.36^{AB}
TT 70	3.03^{A}	ns	1.13^{A}	3.70^{AB}	2.70^{A}	3.91^{A}	1.32^{AB}

Conclusion

At early establishment combinations of PRG sown at 30% or 70% with SRC, WC or TT had little effect on the DM yield of the grass. However, the AA measured in the PRG roots when grown with clover was greater than when no clover was present. Results suggest a beneficial transfer of nitrogen occurred possibly through an interaction between roots. A higher level of ammonium exudation than AA exudation has been found in grass clover experiments, and serine and glycine predominate in exudates from both species (Lesuffleur et al. 2007). However, AA exudates found predominately in grasses are glutamine, glutamate and aspartate but in clovers asparagine predominates (Lam et al. 1996). Surprisingly, there was a higher total concentration of AA in the 70% PRG monoculture treatment than the 30% PRG monoculture treatment. This suggests some same species recognition occurred. This same plant to plant exudate transfer has been observed whereby root development was altered when same, different or own root exudates were present (Dudley and File 2007; Biedrzycki et al. 2010). Whether or by what mechanism the increased AA can contribute to DM yield is yet to be determined. The selection of clovers including both tap rooted and fibrous root types attempted to differentiate between ability to promote DM and AA uptake in grasses. The suggestion that grasses consume exudates from clover roots is not new, but the ratio of clover to PRG to elucidate a response, especially during establishment, is unclear. Differences at the 30% PRG level were significant enough to suggest varying percentages of clover may also elicit a response in AA levels and contributions of short-term N via root exudates. Using less N to grow more pasture at establishment would increases N use efficiency and mitigate against environmental risk.

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