Nutrient distribution among metabolic fractions in 2 *Atriplex* spp.

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Abstract

The seasonal variations in nitrogen and phosphorus fractions and cations in 2 species of *Atriplex* common to Western Australia (*Atriplex amnicola* P. G. Wilson and *Atriplex nummularia* Lindl.) were investigated. Both species contain high concentrations of nitrogen (N) in winter as compared with summer when both species contain high concentrations of sodium. The sum of soluble protein-N, amino acid-N, nucleic acid-N and nitrate-N is about half of the total nitrogen. The remainder includes non-soluble protein-N and other N associated with cell membranes and walls. Phosphorus was more uniformly distributed among pools of inorganic-P, phytate-P, nucleic acid-P and other (residual) fractions. We suggest that interpretation of animal nutrition studies based on similar trichloroacetic acid (TCA) fractionations might be improved by independent estimation of soluble proteins. Fractionation using TCA provides valuable information about the subcellular distribution of both N and P in foliage tissues for studies of plant physiology and animal nutrition.

Concentrations of major nutrients in foliage of both species were significantly and negatively correlated with monthly maximum temperature and significantly and positively correlated with monthly rainfall. In summer and early autumn the apparent nutritive value of both species is well below the basic requirement of sheep or other grazing ruminants such as goats.

Key Words: *Atriplex* spp., saltbush, nitrogen, phosphorus, fractionation, animal nutrition, salinity

Salinity adversely affects the growth and metabolism of many plant species (Greenway and Munns 1980, Flowers et al. 1986, Munns 1993). In arid and semi-arid regions of the world, grasses, herbs and shrubs that can survive in saline soils have been the subject of considerable research. For example, halophytic shrubs have been evaluated as alternative sources of forage for livestock, and *Atriplex* spp. (saltbush) are strong candidates for cultivation in saline areas because of their salt tolerance and productivity (Kleinkopf et al. 1975, O'Leary et al. 1986, Malcolm 1994).

Physiological adaptations of *Atriplex* spp., including the accumulation of high concentrations of sodium and chloride within cell vacuoles and compatible organic solutes in cytoplasm (Cheeseman 1988, Adams et al. 1992), help offset the growth-limiting attributes of soil salinity. Na⁺/H⁺ exchangers or "antiporters" at the tonoplast that can increase Na⁺ accumulation

Resumen

Se investigó la variación estacional de las fracciones de nitrógeno, fósforo y cationes de dos especies de *Atriplex* comunes del oeste de Australia (*Atriplex amnicola* P.G. Wilson y *Atriplex nummularia* Lindl.). En invierno, ambas especies contienen altas concentraciones de nitrógeno (N), comparado con el contenido de verano cuando tienen altas concentraciones de sodio. La suma del N-protéico soluble, N-amino ácidos, N-nucleico y el N-nitratos es aproximadamente la mitad del nitrógeno total. El nitrógeno restante incluye el N-protéico no soluble y el N asociado con las membranas y paredes celulares. El fósforo (P) estuvo más uniformemente distribuido entre las fracciones de P-inorgánico, P-ácido nucleico y otras fracciones (residuales). Sugerimos que la interpretación de los estudios de nutrición animal basados en el fraccionamiento de ácido tricloroacético similar (TCA), pudiera ser mejorada por la estimación independiente de las proteínas solubles. El fraccionamiento utilizando TCA provee valiosa información acerca de la distribución subcelular del N y P en los tejidos foliares, información útil en los estudios de fisiología vegetal y nutrición animal.

Las concentraciones de los principales nutrientes en el follaje de ambas especies fueron significativamente y negativamente correlacionadas con la temperatura máxima mensual y significativamente y positivamente correlacionadas con la lluvia mensual. En verano e inicios de otoño el valor nutritivo aparente de ambas especies es menor que los requerimientos básicos de los ovinos y otras especies de rumiantes tales como los caprinos.

in the vacuole of halophytes, have been suggested as further adaptations to salinity (Staal et al. 1991, Barkala et al. 1995).

The nutritive value of *Atriplex* spp. and other native shrubs for grazing animals has been studied using a variety of techniques (Wilson 1977, Wilson and Graetz 1980, Warren and Casson 1991) which are often based on the work of Van Soest and colleagues (Crooker et al. 1978, Krishnamoorthy et al. 1982, Sniffen et al. 1992, Van Soest 1994). Laboratory procedures for determining nutritive value utilise a range of chemical extractions, especially tungstic and trichloroacetic (TCA) acid in combination with detergents, to fractionate the nitrogen and protein content of plant material into categories which differ in their digestibility (e.g. Licitra et al. 1996). These developments in methodology for studying animal nutrition parallel development of methods for studying plant physiology. For example, Kedrowski (1983) and Chapin and Kedrowski (1983) used a TCA-based fractionation to separate foliar N into classes that differed metabolically and as

sinks for the storage and retranslocation of N both seasonally and with treatment. This method has since been applied to organic-N in litter with similar results (Polglase et al.1992).

Despite methodological advances in both animal nutrition and plant physiology, there is not always an exact relationship between measured attributes of plants and their value to stock. A piece of long-held, and still conventional, wisdom is that protein concentrations are the major index of nutritive value and can be calculated from the concentration of total nitrogen using a 'conversion factor' (e.g. concentration of protein = 6.25 x concentration of Kjeldahl N). On this basis, the National Academy of Science (1958) reported that the concentration of crude protein in *Atriplex* ranges from about 6% to 24 % and others have argued similarly (Beadle et al.1957, Malcolm 1994). While the foliage of *Atriplex* spp. may contain high concentrations of N, there are no definitive studies of the distribution of this nitrogen among classes now commonly accepted in studies of animal nutrition or plant physiology (as identified above). For example, as much as half of total N in *Atriplex* is non-protein nitrogen and of less certain value to grazing animals (Gihad and El Shear 1994).

Further examination of the accumulation of salts and nitrogen fractions in saltbush, and their relationship with soil salinity and climatic factors, are needed before we can accurately evaluate their role as animal fodder. The present study investigated: (a) the relationship between the nutritive value of foliage of 2 *Atriplex* spp. and environmental factors, such as rainfall and temperature; and, (b) the distribution of total nitrogen and phosphorus (elements that limit growth in Western Australian soils) among discrete 'fractions' in the foliage. We compared 2 perennial species, *A. amnicola* P. G. Wilson (River saltbush) and *A. nummularia* Lindl.(Old man saltbush).

Materials and Methods

Study sites

Field work for this study was conducted between February 1995 and January 1996 at a 10 ha site close to Tammin (31° 39' S, 117° 29' E; 200 km east of Perth, Western Australia). The site has been previously described by Davidson et al. (1991). Briefly, the soil is saline (conductivity of 60–80 ds/m) and has a high sodium absorption ratio (SAR) 80–100. The surface soil horizon lies above a dense clay B horizon and may become waterlogged after

rain. The 2 species of saltbush have been grown side by side on the same site for more than 18 years. The species were planted in alternate rows on a 2.5 x 2.5 m grid along a topographic gradient of soil salinity (from low to high salinity).

The climate of the study area is semi-arid, with considerable seasonal and daily variations in temperature. Precipitation and temperature data were obtained from meteorological stations at Tammin and Cunderdin, less than 25 km away from the study site. Mean maximum temperature for the hottest month during the study (January) was 34°C and the mean minimum for the coldest month (July) was 7°C (Fig. 1a). Total precipitation during the study period was 437 mm and almost half of that fell between June and August (Fig. 1b). These values are all close to the long term means for Tammin, and evaporation exceeds precipitation for much of the year.

Sampling

Leaf samples were collected from 6 randomly selected plants of *A. amnicola* and *A. nummularia* at monthly intervals starting in February 1995 along the topographic salinity gradient. Leaf samples were immediately plunged into liquid N₂ and freeze-dried on return to the laboratory. All analyses were conducted on freeze-dried tissues and were made in duplicate. Mean values were used for statistical analyses.

Chemical analysis

Total nitrogen and phosphorus concentrations were measured by digestion of dried and ground leaf samples in H₂SO₄/H₂O₂ at 320°C (Adams and Attiwill 1986) and subsequent colorimetric determination of PO₄³⁻ and NH₄⁺. The method of Murphy and Riley (1962) was used to measure PO₄³⁻ and the indophenol

Fig. 1. Climatic conditions at Tammin, Western Australia, from February 1995 to January 1996. (a) Monthly maximum, minimum and mean ambient temperatures. (b) Monthly rainfall.

Fig. 2. Seasonal pattern of N fractions in (a) *A. amnicola* and (b) *A. nummularia*. Least significant differences for $p = 0.05$ (LSD) between months for each fraction are shown.

blue method of Keeney and Nelson (1982) was used to measure NH_4^+ .

Leaf N and P were fractionated using sequential extraction with cold (room temperature, 0.3 M) and hot (90°C, 0.15 M) trichloroacetic acid (TCA) by the procedure of Polglase et al. (1992) modified from Chapin and Kedrowski (1983). Usually, extracts would be analysed for inorganic and total N and P and the concentration of organic N and P calculated as the difference. Concentrations of inorganic N in cold TCA extracts were negligible and we assumed that the total N was derived largely from amino acids (Chapin and Kedrowski 1983). Similarly, hot TCA extracted mainly organic N (assumed to be nucleic acids) and there was little inorganic N present.

Inorganic phosphorus was present only in cold TCA extracts. Organic phosphorus

(P_o) soluble in cold TCA was assumed to be a combination of phytate P and other ester P, and P_o soluble in hot TCA to be a combination of phytate P and nucleic acid P. The sum of P and N fractions in cold and hot TCA extracts we defined as labile P and labile N (Polglase et al. 1992). Residual components were defined as those insoluble in TCA.

Nitrate (NO_3^-) concentrations in foliage were measured separately by the extraction and analysis procedures described by Cataldo et al. (1975). The total concentrations of Na^+ , K^+ , Ca^{2+} and Mg^{2+} were determined by digestion of leaf samples in acid (as described above for analysis of total N and P) followed by atomic absorption spectroscopy (e.g. Adams and Attiwill 1986).

Soluble protein was determined by a modified procedure of Wilkins et al. (1994) with 50 mg of leaf tissues homogenised with 1.2 ml of 100 mM Tris /HCl pH 7.6, containing 1.5 mM disodium ethylenediamine tetraacetic acid (EDTA), 1.5 mM MgCl_2 , 1.5 mM KHCO_3 , 2.5 % (v/v) Tween 20, 10 % (v/v) glycerol, 100 mg of sand and 10 % (w/v) insoluble polyclar AT. The crude extracts were centrifuged at 15,000 g for 5 minutes, decanted and the supernatant re-centrifuged for 2 min at 15,000 g. The clear extract was analysed for protein using the method of Lowry et al. (1951) with a kit purchased from Bio-Rad.

Statistical analysis was performed using the General Analysis of Variance procedure available in the Genstat computer package. Species and season were considered the independent variables. Harvest dates were treated as repeated measures in time and analysed using a randomised complete block design.

Fig. 3. Concentrations of soluble protein in foliage of *A. amnicola* and *A. nummularia*. The least significant difference for $p = 0.05$ (LSD) between species for all seasons is shown.

Results

Nitrogen

Concentrations of amino acid nitrogen (soluble in cold TCA) and nucleic acid nitrogen (soluble in hot TCA) for both species varied throughout the year (Figs 2a, 2b). In particular, concentrations of amino acid nitrogen were greatest in summer and early autumn (18–31% of total nitrogen) and least in winter and spring (5–14% of total nitrogen). Concentrations of nucleic acid nitrogen varied less (between 6 and 14%) and little seasonal pattern was apparent. Separate analysis of nitrate concentrations in foliage of both species showed little seasonal trend but were greater during periods of rainfall (6–11%) than at other times (2–4%). The concentrations of total nitrogen in leaves of both species were greatest in winter months and least in summer. The difference between total N and labile N (nucleic acid N + amino acid N + nitrate N) we defined as residual N and comprised by far the largest N fraction throughout the year (Figs 3a, 3b).

Further estimates of the forms of N in foliage were obtained using a direct extraction technique for soluble protein. On a seasonal basis (Fig 3), concentrations of soluble protein were significantly greater in winter than in other seasons (on the basis of the ratio of protein:N of 6.25, the approximate concentration of N in soluble protein varied between about 0.9 and 1.2 mg N g⁻¹ dry weight). Seasonal mean concentrations of total N and their distribution into residual and labile N (Fig 4) followed a similar pattern to those of soluble protein. The concentration of total N was significantly less in *A. nummularia* than in *A. amnicola* throughout the year ($p < 0.01$).

Phosphorus

Phosphorus was more evenly distributed among fractions than was nitrogen (Figs 2a, 2b, 5a, 5b). Most fractions followed

Fig. 4. Residual and labile N in *A. amnicola* (black and white) and *A. nummularia* (shaded). The least significant difference for $p = 0.05$ (LSD) between species for total N for all seasons is shown. The proportion of labile-N as % is given in brackets.

closely the pattern of rainfall (Fig 1b). Inorganic P comprised up to 60% of total leaf P in summer while phosphorus fractions soluble in hot TCA (phytate P plus nucleic acid P) increased to close to 40% of the total in winter. In contrast to nitrogen, residual phosphorus was the smallest fraction in both species throughout the year (Fig 6). Combination of monthly data into seasons shows clearly that total P concentrations were greatest in winter for both species and greater in *A. amnicola* than in *A. nummularia* ($p < 0.01$).

Cations

In *A. amnicola* and *A. nummularia*, the concentration of Na⁺ was about 4.5% in mid-winter, and increased to maximum of 7–8% by mid-summer (Fig 7a, 7b). In both species the concentration of K⁺ increased in winter to 3% for *A. amnicola* and 3.6% for *A. nummularia*. The concentration of K⁺ was significantly ($p < 0.01$) greater in *A. nummularia* than *A. amnicola*

during most of the year. In both species, the Na:K ratio in summer and autumn was greater than 3:1 compared with winter and spring when the ratio approached 1–1.5:1.

In *A. amnicola*, the concentration of Mg²⁺ in summer and autumn was 0.16%, which increased to 0.21% in spring and winter. In *A. nummularia*, the concentration of Mg was about 0.10% in summer and autumn and 0.13% in winter and spring. In both species, the concentration of Ca²⁺ slightly increased in late autumn and winter (0.99%), while remaining constant throughout the growing season.

Correlation

The concentrations of total nitrogen, nitrate, total phosphorus and potassium were significantly and negatively correlated with monthly mean maximum temperature ($p > 0.05$) and significantly and positively correlated with monthly rainfall ($p < 0.05$). By contrast, sodium concentration was significantly and positively correlated with maximum mean monthly tem-

Table 1. Pearson correlation coefficients between environmental factors and concentrations of nutrients in *Atriplex amnicola* and *Atriplex nummularia*.

	<i>Atriplex amnicola</i>			<i>Atriplex nummularia</i>		
	Max. Temp	Min Temp	Rainfall	Max. Temp	Min Temp	Rainfall
Total Nitrogen	-0.86**	-0.87**	0.59*	-0.73**	-0.74**	0.67*
Nitrate (NO ₃)	-0.60*	-0.58*	0.72**	-0.69*	-0.66*	0.73*
Total Phosphorus	-0.87**	-0.86**	0.77**	-0.72**	-0.73**	0.67**
Sodium	0.94**	0.90**	-0.72**	0.83**	0.65*	-0.65*
Potassium	-0.75**	-0.66*	0.72**	-0.84**	-0.78**	0.61*
Calcium	-0.46NS	-0.34NS	0.41NS	-0.71**	-0.79**	0.45NS
Magnesium	0.40NS	-0.48NS	0.16NS	-0.63*	-0.64*	0.26NS

Significance * $p < 0.05$, ** $p < 0.01$, NS = not-significant at $p = 0.05$.

Fig. 5. Seasonal pattern of P fractions in (a) *A. amnicola* and (b) *A. nummularia*. Least significant differences for $p = 0.05$ (LSD) between months for each fraction are shown.

perature ($p < 0.01$) and negatively with total monthly rainfall (Table 1). In both species, throughout the whole growing season, there were highly significant positive correlations between nitrogen and phosphorus concentration ($p < 0.01$) and potassium concentration was negatively correlated ($p > 0.01$) with sodium concentration.

Discussion

Little, if any, attention has been given to the metabolic fractions of N and P in native Australian shrub species or indeed for many other, non-agricultural, trees and shrubs (Chapin and Kedrowski 1983). By far the greatest proportion of total N in *Atriplex* was present in the residual fraction that includes the soluble and non-soluble proteins and cell wall components and which varied considerably throughout the year. Amino acid-N, nucleic acid-N and

nitrate-N were minor fractions showing little seasonal variation. Separate analysis suggested that the N-content of soluble protein was of the order of 1 mg g^{-1} and hence the majority of the residual -N fraction of 10–18 mg g^{-1} is present as insoluble protein (probably associated with cell or organelle membranes) or cell wall components. The conflicting reports of digestibility and nutritive value for grazing animals of shrubs provide little by way of comparative data for the present study. Indeed, Le Houérou (1992) reported that the "feed value of *Atriplex* spp. has been questioned by a number of scientists and still is a controversial issue". Wilson (1977) assessed nitrogen fractions in a range of shrubs found in western New South Wales using methods based on acid and detergent fractionation. Wilson

argued that the true digestibility (in sheep) of organic matter of 2 *Atriplex* species (including *A. nummularia*) was about 75%, that of neutral-detergent fibre about 60%, of acid-detergent fibre about 40% and of nitrogen about 95%. In contrast, Le Houérou (1992) summarised a range of other studies in claiming a digestibility of nitrogen in *Atriplex* of only 65% and in addition pointed out that only about half of that was retained. The N not retained was largely glycinebetaine—a solute which accumulates during drought and especially salinity stress. In our study, glycinebetaine would have been included in the amino acid-N fraction which was always small and while glycinebetaines may reach micro-molar concentrations in salt-tolerant trees (Prat and Fathi-Ettai 1990) or shrubs under drought or saline conditions (Singh et al. 1973, Cyr et al. 1990, Storey et al. 1993, Kozłowski and Pallardy 1997) it is unlikely that this or other osmotically active nitrogenous solutes (e.g. proline) comprise a significant proportion of digestible-N in *Atriplex*. The increases in concentration of amino acid-N during summer were not reflected in total-N which declined possibly due to a) inhibition of nitrate reductase activity by water and salt stress (Hsiao 1973, Kleinkopf et al. 1975), b) $\text{NH}_4^+/\text{Na}^+$ competition (Naidoo 1987) or c) competition for sites of NO_3^- uptake by Cl^- on the basis that Cl^- is a major osmoticum in halophytes (Cram 1973).

Our finding that N in non-soluble protein or cell-walls is by far the largest proportion

Fig. 6. Residual and labile P in *A. amnicola* (black and white) and *A. nummularia* (shaded). The least significant difference for $p = 0.05$ (LSD) between species for total P for all seasons is shown. The proportion of labile-P is given in brackets.

Fig. 7. Seasonal pattern of cation fractions in (a) *A. amnicola* and (b) *A. nummularia*. Least significant differences at $p = 0.05$ (LSD) between months for each cation are shown.

portion of the largest N-fraction (residual-N) which in turn strongly reflects seasonal fluctuations in growing conditions is consistent with other studies (Chatterton et al. 1971) and with the general physiological pattern that up to half of leaf N is associated with the membrane-associated enzymes of carbon fixation (e.g. RUBISCO, Field and Mooney 1986). It seems axiomatic that studies of plant N from the perspective of animal nutrition might benefit from a better understanding of the digestibility and retention of this N source.

Phosphorus, which has a central role in the energy metabolism of grazing animals as well as plants, was also lower in concentration in both species during summer and early autumn (Grice and Muir 1988) than recommended for grazing animals (0.16–0.37%, National Research Council, 1975), but was adequate during the rest of the year. In summer, the concentration of inorganic phosphorus was greater than that

of other P-fractions in both species. Inorganic-P acts as a P-storage pool in the vacuole and can be utilised during the summer period of intense growth (Marschner 1986). In winter, the increase in the phytate and nucleic acid P-fraction coincided with a comparable decline in inorganic phosphorus suggesting that P-storage is a major process during the colder months.

Both *Atriplex* species contain considerably greater Na^+ concentrations than that recommended as being suitable for ruminants (0.06% National Research Council 1981). In summer, both species contain more than 6% by weight of Na^+ . These high concentrations make *Atriplex* a poor quality forage and increase the demand of stock for good quality water (Grice and Muir 1988). Again, K^+ concentrations in both species were greater than those recommended by the National Research Council (1975, 1981 0.5–0.8%). Low con-

centrations of salts in foliage during winter may be a result of leaching from epidermal trichomes (salt bladders) which contain at least 50% of salt of the leaves (Pallaghy 1970).

In recent years, *Atriplex* spp. have increasingly been recognised as poor fodder for stock because of their high concentrations of salts and other metabolites, as found here, and due to the small proportion of total N present in readily digestible forms. In arid and semi-arid areas of developing countries, supplementary feeding will be necessary to overcome deficiencies in *Atriplex* as a stock feed (e.g. Le Houérou 1992). However, our results indicate that there is a substantial variation in the potential forage value throughout the growing season and this variation could be used to increase the value of saltbush for animals at specific times of the year. Clearly, any measure of "nutritional value" based on estimates of total N and thus "crude protein" are inaccurate. Notwithstanding, the fermentation and digestion capability of ruminant animals, alternative measures of the availability of N in foliage, may help in assessing the value of forage species in rangelands (and elsewhere).

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