Predicting Nitrogen Deficiency in *Eucalyptus nitens* Plantations Using Soil Analysis and Budgeting Methods

by

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In Memory of

Frederick and Gertrude Moroni

Declarations

This thesis contains no material which has been accepted for a degree or diploma by the University of Tasmania or any other institution. To the best of my knowledge and belief this thesis contains no material previously published or written by another person except where due acknowledgment is made in the text of the thesis.

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Abstract

Areas of *Eucalyptus nitens* plantations in Tasmania are increasing and are expected to enhance Australia's production of wood products. Standard silvicultural practises involve N fertilisation of *E. nitens* plantations at planting and later ages, however not all plantations respond to N fertiliser applied either at planting or at a later age. A method to predict N fertiliser responses is required to prevent wastage and obtain maximum productivity of Tasmanian *E. nitens* plantations. In this study nitrogen budgeting and soil analysis methods were examined as predictors of the timing of N fertiliser responses.

Fourteen established research localities within Tasmania were used for the study, covering a wide range of sites planted to *E. nitens*. Of the 14 sites, 11 were on basalt, with single representatives on siltstone, granite, and alluvium. Rainfall ranged from 1039-1913 mm per annum and elevation ranged from 170 m to 650 m. Sites were variously fertilised, some at planting, others at growth stages up to ten years.

Net nitrogen mineralisation (NNM) was estimated *in situ* at five sites encompassing a wide range in N fertility. NNM in these sites ranged from 13 to 188 kg N ha^{-1} $year^{-1}$. Soil analyses for total N, total P, total C, hot KCl extractable N, soil solution and cold KCl extractable N were examined as indices of NNM. Total N, total P, total C, and hot KCl extractable N did not show large temporal variation and the values attributed to these indicators separated the five sites into two groups, being sites with NNM greater or less than 40 kg N ha^{-1} $year^{-1}$. Sites of NNM >40 kg N ha^{-1} $year^{-1}$ had total N greater than 0.4%, total P greater than 0.2%, total C greater than 8% and hot KCl extractable N greater than 100 μg N g $soil^{-1}$. Sites with lower NNM had concomitantly lower values of these soil analyses.

The biomass of tree components was estimated from pre-determined regressions with tree size. Measurements or estimates of N concentrations led to estimates of N content in tree components at 14 sites. Nitrogen content of litterfall was estimated at two sites with high soil N analysis values, one of known high *in situ* NNM rates. Maximum

estimated N uptake in the combined above-ground biomass, the below-ground biomass and the litterfall was 162 kg N ha^{-1} $year^{-1}$.

Fertiliser responses were deemed to be significant ($P<0.05$) when increments in stem diameter at breast height (1.3 m) over bark of fertilised trees was significantly greater than diameter increments of unfertilised trees. For sites fertilised at planting the initial year of significant response was recorded, while for sites fertilised at a later age (age 3-10 years) relative responses (diameter increment of fertilised trees / diameter increment of unfertilised trees) were recorded. Of sites fertilised at planting, two had responded by age two years, one by age three years and three had not responded by age three years. Relative responses of sites fertilised at a later age ranged from 99% to 171%.

The formation of a simpler partial budget, where N supply was only *in situ* NNM (0-10 cm depth; 5 sites) and N demand was only the N increment into the above-ground biomass, was always able to predict a significant response to fertiliser. However, when NNM was estimated with soil analyses, fertiliser responses were accurately predicted in only five of 14 sites.

The six sites fertilised from planting could be separated, on the basis of total soil N, into those that responded [significant ($P<0.05$) increase in stem diameter at breast height in N fertilised trees compared to unfertilised trees] before age three years (n=2), at age three years (n=1) and after age three years (n=3) with total N of <0.28%, 0.28-0.51% and >0.51% respectively. All sites that responded to N fertiliser had soil solution and cold KCl extractable nitrate below 0.1 mM and 1 μg N g^{-1} soil respectively. The combination of total N and soil-solution or cold KCl-extractable nitrate allowed prediction of N fertiliser responses at all of the 14 study sites.

Both soil analysis and one budgeting method were successful in predicting N fertiliser responses of *E. nitens* plantations. The budgeting method was most successful when NNM was estimated *in situ*, which together with the tree measurements required, is labour- and time-intensive. Unless estimation of NNM and N uptake can be simplified, the budgeting technique is unlikely to become part of standard silvicultural

practices. A soil analysis method using total N and soil solution or cold KCl extractable N is simpler and more likely to be used by forest managers.

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TABLE OF CONTENTS **Page**

Page

1. INTRODUCTION

Australia had a deficit in wood and paper products in 1997-98 to the value of Aust $1.6 billion or 0.35% of Gross National Product (Australian Bureau of Statistics 1988a and 1988b). Expansion of Australia's plantation estate is being promoted by the federal government and the forest industry as an essential part of measures needed to address this problem (Australia, Forest Taskforce 1995; Plantations for Australia: the 2020 vision 1997). This policy framework is matched by commercial and environmental incentives for investing in plantations. Australia has approximately 1.04 million ha of plantations, of which 0.16 million ha (15%) are planted with hardwood species (mostly *Eucalyptus* spp.). About 40% of these hardwood plantations have been planted in Tasmania, predominantly as *Eucalyptus globulus* and *Eucalyptus nitens,* grown essentially for pulp and paper production. *Eucalyptus* has replaced *Pinus* as the main genus planted both nationally and in Tasmania. For example, during 1990-94 eucalypts represented over 80% of the new-planted area (National Forest Inventory 1997).

In the north-west of Tasmania, North Forest Products (NFP) currently manage 43 000 ha planted to *E. nitens* (90%) and *E. globulus* (10%). Projected planting rates for the next few years are 6 500 ha year^{-1} of *E. nitens* (pers. com. David DeLittle). Approximately similar areas of *E. nitens* or *E. globulus* are expected to be planted on other soil types throughout the state by NFP and other growers. These plantations are expected to significantly improve Australia's balance of payments in wood products during the next two decades.

Conversion of native forest to plantation increases pulp yield, resulting in industry preference for plantation wood chips. For example, in Tasmania, North Forest Products receive a 10% premium on plantation chips over native forest chips. The greatest economic benefits for plantations grown for pulp come, in decreasing order, from increases in growth rate, wood density and pulp yield. Plantation *E. nitens* tends to have lower density than those in native forest, however this is offset by increases in volume. The lower density of plantation trees usually results in better fibre collapse, better inter-fibre bonding and therefore improved fibre strength. (Downes *et al.* 2000,

and 1997; Greaves *et al.* 1997). Hence, it is generally desirable to attain maximum growth rates from plantations, leading to selection of more fertile sites and fertiliser addition.

The majority of these *E. nitens* plantations have been planted between 300 m and 700 m elevation on basalt-derived soils (Holz pers. comm.) i.e. Ferrosols (Isbell 1996). The growth of many *Eucalypt* plantations on these and a variety of other soils in Tasmania and Victoria is probably limited by the low availability of nitrogen (N) (Bennett *et al.* 1997; Cromer *et al.* 1993; Holz pers. comm.). For example application of N to three *E. globulus* plantations in Victoria during ages 2-26 months increased under-bark volume by 29-55% by age six years (Bennett *et al.* 1997). Also in Tasmania, many plantations have responded to N added soon after planting and at later ages. For example five *E. nitens* plantations in northern Tasmania responded to N added at ages 3-6 years (Holz pers. comm.).

Most soil N is in an organic form that cannot be taken up by plants unless it is mineralised to form NH_4^+ or subsequently nitrified to form NO_3^-, both of which are available for uptake from the soil solution (Engels and Marschner 1995). Ammonium and NO_3^- mineralised from soil organic matter (SOM) form the bulk of N available for plant growth in unamended soils (Raison and Stottlemyer 1991; Sierra 1992; Campbell *et al.* 1994; Pastor *et al.* 1984) with *E. nitens* preferentially assimilating NH_4^+ (Garnett 1996). A portion of the gross N mineralised is immobilised by the soil microflora leaving a net amount available for uptake (Singer and Donald 1996; Tisdale *et al.* 1993). When net N mineralisation (NNM) is insufficient to meet the demand for N by a plantation, a growth response to added N is expected. An ability to estimate when this will occur would allow forest managers to apply N as it is required for growth, thereby increasing the efficiency of N-fertiliser usage and reducing the potential for leaching of N into groundwater and streams.

Soil disturbance, such as those involved with site preparation and planting, is expected to temporarily raise rates of NNM. Hence, it is expected that rates of NNM will decline with time after planting, as has been observed in other studies. For example, in South Australian *P. radiata* plantations rates of NNM more than halved from the first

to the third year after planting (Smethurst and Nambiar 1990a). Thus decreasing rates of soil N supply may require increasing N application to maintain stand productivity.

In-situ rates of NNM measured in two Tasmanian plantation soils have been ranked in the same order as the concentration of total N or mineralisable N extracted during anaerobic incubation and hot KCl extraction (Wang *et al.* 1996a). These analyses have been related to N availability, growth and yield of other crops (Keeney 1982; Binkley and Hart 1989). For example Shumway and Atkinson (1978) demonstrated significant relationships between N released during anaerobic incubation and the growth response of douglas fir to applications of N fertiliser. Hence, two hypotheses can be tested; that soil analyses, those of Wang *et al.* (1998) in particular, will correlate with *in situ* NNM, i.e. N supply, or directly to the response of *E. nitens* plantations to N fertiliser application.

Most studies of N uptake and tissue N cycles in eucalypts are in native forest. Of the studies in plantations, few are in *E. nitens* and none have been published for plantations in Tasmania. Measurement of N fluxes to and from the biomass of Tasmanian *E. nitens* will increase our understanding of N requirements, may help predict rates of NNM and may be used to predict N deficiency by way of an N budget.

Estimates of annual N uptake into the above-ground tissues of the sub-genus *Symphomyrtus (E. globulus* and *E. nitens)* reported in the literature are as high as 120 kg for a 3-year-old *E. globulus* plantation in Portugal (Pereira *et al.* 1996). The supply of mineral N from NNM in Tasmanian soils supporting *E. nitens* plantations was estimated by Wang *et al.* (1998) to range from 18 to 91 kg N ha^{-1} $year^{-1}$ (0-10 cm depth). Sites represented by the lower part of this range are unlikely to provide adequately for N-uptake of fast-growing plantations. Given information on NNM and subsequent N losses from soil, it is evident that estimates of annual N uptake within *E. nitens* plantations should provide an estimate of when NNM is sufficient or otherwise to meet the N requirements of tree growth at particular sites.

Hence, the principal research objective of this thesis was to evaluate soil analyses and budgeting methods as indicators of N fertiliser requirements to maximise growth of Tasmanian *E. nitens* plantations.

To achieve this objective, 14 sites planted to *E. nitens,* of ages 1-10 years, each containing an N fertiliser experiment with a history of different N fertiliser applications were selected for study. For the development of a partial N budget, NNM was compared to N demand (N uptake from the soil). Soil *in situ* NNM (0-10 cm) was estimated for two years at five of these sites. Soil analyses were correlated to these *in situ* rates of NNM and subsequently used to estimate NNM at the remaining nine sites. Nitrogen uptake was estimated for 12 of these sites from pre-determined biomass regression equations based on diameter and height and for the remaining two sites from destructive sampling conducted by Cromer *et al.* (unpublished). Nitrogen in leaf, branch, and bark litter was measured at two of these sites (ages three and ten years).

Within each experimental site, where stem diameters or increments in stem diameter at breast height of fertilised trees were deemed significantly greater ($P < 0.05$) than those of unfertilised trees, a growth response was recorded. For trees fertilised within one year of planting, the age when a significant response in diameter growth to added N was recorded. For trees fertilised at a later age, relative responses were recorded. Relative responses are diameter (1.3 m) increment of fertilised trees/diameter (1.3 m) increment of unfertilised trees.

2. SURFACE SOIL N FLUXES IN FIVE TASMANIAN *E. nitens* PLANTATIONS

2.1 INTRODUCTION

Rates of NNM limit the growth of many of the world's forests. For example, Pastor *et al.* (1984) and Nadelhoffer *et al.* (1985) have shown that rates of N mineralisation were correlated with above-ground productivity of several temperate native North American forests. Raison *et al.* (1992) found annual N uptake of a ten-year-old pine plantation in the Australian Capital Territory was limited to the sites NNM rates. In Tasmania's native forests of *E. obliqua* and *E. amygdalina,* Adams *et al.* (1989b) found rates of NNM were positively correlated with productivity measured as the product of tree height and basal area. Tasmanian *Eucalypt* plantations are known to respond in diameter and height to added N (G Holz pers. comm), indicating NNM is unable to satisfy N demands for fast growth. Of relevance, NNM in podzolised sands (0-15 cm) of South Australia was sufficient to meet N uptake estimates of fast growing *P. radiata* for the first two years. Thereafter, estimates of potential uptake exceeded NNM and it was suggested that applications of fertiliser would be effective at that stage of the crop growth (Smethurst and Nambiar 1990a and 1990b). The suggestion that applications of fertiliser would be effective at that stage of crop growth was later confirmed (Fife *et al.* 1995).

Rates of NNM are affected by a number of soil and environmental factors. For example, soil temperature and water strongly affect rates of NNM (Stanford *et al.* 1973; Vigil and Kissel 1995; Jensen *et al.* 1997; Gonclaves and Carlyle 1994; Campbell *et al.* 1981). Generally, microbial activity follows a Q_{10} of 2.0 between 5 °C and 35 °C (Rice and Havlin 1994; Stanford *et al.* 1973; Vigil and Kissell 1995). At a given temperature, rates of NNM increase with increasing water content, as long as aerobic conditions are maintained (Quemada and Cabrera 1997; MacDonald *et al.* 1995; Schepers and Messinger 1994). If soil water content becomes too high, soil anoxia develops, rates of NNM and nitrification decrease, and those of denitrification increase (Carter and Rennie 1982).

Both the quantity and quality of organic N affect rates of NNM (Boone 1992; Jensen *et al.* 1997; Gonclaves and Carlyle 1994; Van Praag and Weissen 1973). Readily degraded fractions of organic N often form a small portion (1-3%) of the total N pool (Bremner 1965), but they are the source of most N mineralised (Christenson and Butt 1997; Jarvis *et al.* 1996; Cabrera *et al.* 1994; Semyonov 1996). Organic matter may be physically protected from mineralisation, being enclosed by or part of large tissues (Rice and Havlin 1994; Jarvis *et al.* 1996), it may be adsorbed to negatively charged clay surfaces that effectively coat it (Oades 1988), or be located within small pores of micro-aggregates physically isolating it from the biosphere (Tiessen and Stewart 1983; Christensen and Sorensen 1985). Soil disturbance, such as cultivation during plantation establishment, exposes new OM surfaces and breaks some physical protection leaving fresh organic matter surfaces accessible for mineralisation by soil microbiota, temporarily increasing rates of NNM (Clay *et al.* 1995; Connell *et al.* 1995; Jarvis *et al.* 1996; Binkley and Hart 1989; Raison *et al.* 1987). Hence, rates of NNM commonly decrease during the first few years after plantation establishment. For example, in South Australia, Smethurst and Nambiar (1990a) found a decrease in NNM rates from 50-70 kg ha^{-1} during the first year to 20-30 kg ha^{-1} during the third year after planting of *P. radiata*. Raison *et al.* (1992) found a decrease in NNM rates from 38 kg N ha^{-1} $year^{-1}$ to 7 kg N ha^{-1} $year^{-1}$ over a four year period in a *P. radiata* plantation. Such patterns are also likely to occur in *Eucalypt* plantations. Hence, within the study sites *in situ* rates of NNM are expected to decrease with time.

Mineralisation of OM produces the NH_4^+ cation, which is nitrified producing the NO_3^- anion. Soils generally have a large cation exchange, where negative soil surfaces attract a balancing cation, holding it against leaching, while maintaining its availability to plants. Negative surfaces can be associated with inorganic compounds (sand, silt and clay), OM and roots (Tisdale *et al.* 1993). Within clay, comprised largely from layered silica and aluminum compounds, the major source of negative charge arises from replacement of Si^{4+} or Al^{3+} with cations of lower charge (isomorphic substitution). Variable charge surfaces occur in OM, and in clay where Al-OH and Si-OH groups occur on the broken edges of 1:1 layered minerals such as kaolinite and where oxy hydrous Fe and Al occur in the clay fraction, common in kaolinitic clays. Deprotonation of these groups contributes to cation exchange

capacity of soils. Within ferrosols the primary clay is kaolinite, which has far less isomorphic substitution and hence a much lower cation exchange than other clays, for example less than 1% of montmorillonite. As a result the cation exchange capacity of kaolinite is often predominantly associated with OM surfaces. Protonation of the relatively large amount of variable charge groups associated with kaolinite contributes to an appreciable anion exchange capacity of ferrosols. However, of greatest significance to ferrosols anion exchange is the protonation of carboxyl groups associated with OM (Moody 1994; Tisdale *et al.* 1993). Anion exchange is generally low in surface soils, increasing in the subsoil (Black and Waring 1976, Gillman and Abel 1987), hence anion exchange is likely to have little effect on NO_3^- movement in the 0-10 cm study depth, but may hold significant amounts of NO_3^- at depth.

Estimates of field rates of NNM require *in situ* assessment because the environmental effects on the process can not be easily accounted for in the laboratory. Sequential *in situ* soil core methods also avoid the effects of soil disturbance required by some laboratory incubation methods. Early field incubations used steel cans with perforated walls that were capped at the top and bottom (Lemee 1967). Because perforated sides may result in the loss of NH_4^+ and NO_3^- by mass flow and diffusion (Hart *et al.* 1994), unperforated walls have been used in many subsequent studies. Variations of these methods include the use of buried disturbed soil (e.g. Westermann and Crothers 1980; Vitousek and Matson 1985) or undisturbed soil (e.g. Nadelhoffer *et al.* 1984, 1985; Matson and Boone 1984) in plastic bags, of ion exchange resins in open soil cores (e.g. Binkley and Matson 1983; Hart and Binkley 1985), or the use of capped metal cans (e.g. Rapp *et al.* 1979) or PVC tubes pushed into the soil surface to isolate a soil column (e.g. Adams and Attiwill 1986a). Raison *et al.* (1987) preferred *in situ* sequential coring because it minimised soil disturbance and was easier than buried bag techniques or the use of ion exchange resins. Raison *et al.* (1987), Kolberg *et al.* (1997) and Adams *et al.* (1989a) validated the *in situ* soil coring technique as satisfactory for quantifying fluxes of mineral N in the field. Recommended durations of incubations range from 30 days to 90 days (Goncalves and Carlyle 1994; Carlyle 1995a; Smethurst and Nambiar 1989; Carlyle *et al.* 1998; Kolberg *et al.* 1997). The duration of incubation *in situ* should be sufficient to measure a significant change in the concentration of inorganic N, but also be short enough to minimise time-

dependent differences in water, temperature, and microbial processes that develop between soil inside and soil outside the cores (Smethurst and Nambiar 1989a; Raison *et al.* 1987; Edmonds and McColl 1989; Adams *et al.* 1989a). Potential errors involved with application of this technique come from severing of fine roots during insertion of the tubes, providing additional substrate for mineralisation, and subsequent temperature and moisture differences that may be generated between soil inside and outside the tube, possibly altering NNM rates (Raison *et al.* 1987).

Tree root distribution is greatest near the surface, decreasing with depth. For example, root intercepts per 200 cm^2 (vertical plane) of soil in Tasmanian *E. nitens* dropped from 21-0 counts from the 0-10 cm depth to the 80-90 cm depth in droughted trees and from 20-0 counts from the 0-10 cm depth to the 50-60 cm depth in irrigated trees (Moroni *et al.* 1999). Similarly, for a 34 month old Tasmanian *E. nitens* plantation Misra *et al.* (1998a) report decreases in fine root (< 1 mm diameter) density from 0.3 kg m^{-3} in the 0-10 cm depth to less than 0.1 kg m^{-3} within the 30-45 cm depth and decreases in medium root density (1-3 mm diameter) from 0.2 kg m^{-3} in the 0-10 cm depth to less than 0.01 kg m^{-3} below 45 cm depths. Rates of NNM are also greatest in surface soils. For example, an average of 32% of the N mineralised in the top 20 cm of soil under a wheat crop originated in the 0-2 cm layer (Purnomo *et al.* 2000) and in soil from cereal crops of South Australia, mineralisable N in the 0-10 cm depth accounted for 90% of total mineralisable N in the 0-20 cm soil depth (Xu *et al.* 1996). Hence, soil sampling was limited to the 0-10 cm depth of the Wang *et al.* (1998) study. However, greater depths have been sampled during other studies (for example 0-20 cm with *in situ* studies Raison *et al.* 1987; O'Connell and Rance 1999 and with soil analyses such as aerobic incubation Aggangan *et al.* 1998 and 0-30 cm *in situ* studies Smethurst and Nambiar 1989b). Roots penetrate the soil to much greater depths, e.g. Tasmanian *E. nitens* to 80 cm at 34 months (Misra *et al.* 1998a). Hence NNM below 10 cm may contribute significantly to N supply. Contributions of the subsoil to NNM are examined in chapter 3.

The study sites were strip cultivated producing a mound approximately 30 cm in height, into which the seedlings were planted. This had the effect of preparing the soil for planting and raising the seedlings from the coldest winter air at ground level,

reducing frost damage. The cultivation producing the mounds inverted, rotated and mixed soil and soil horizons. The mounded soil was thus more heterogeneous than uncultivated soil. Hence errors of measurement in cultivated soil were expected to be greater than in uncultivated soil, therefore measurements were concentrated in uncultivated soil. The effect of cultivation is expected to further increase rates of NNM in the cultivated strips.

Rates of NNM were estimated at four sites (3 ex-native forest, 1 ex-pine) supporting *E. nitens* in Tasmania by Wang *et al.* (1998). Rates of NNM were measured for a further two years at these sites to examine the hypothesis that rates of NNM would decrease with time at these sites. An ex-pasture site was also included in the study. Many pasture and cropped lands are fertilised and include N-fixing legumes that add organic N. Dead plant materials and animal excreta can also contribute to soil N and mineralisable N (Whitehead 1995). Wang *et al.* (1996a) found higher concentrations of anaerobically mineralisable and hot KCl extractable N in soil from ex-pasture sites when compared to ex-native forest and ex-pine sites. Hence the ex-pasture site was expected to have high NNM rates. In part because of their high fertility, ex-pasture sites are highly sought after for establishing forest plantations.

In surface soils of five *E. nitens* plantations several hypotheses were tested: (1) *In situ* rates of NNM and nitrification would be higher at an ex-pasture site than at ex-native forest and *P. radiata* sites. (2) Ranking of these fluxes would remain consistant across several years. (3) Rates of NNM, nitrification, leaching and concentration of mineral N would decrease during the first few years of plantation establishment. (4) Rates of NNM would be higher in cultivated strips than between these strips.

2.2 MATERIALS AND METHODS

2.2.1 Site description

Wang *et al.* (1998) reported N fluxes at one ex-pine (Boulder) and three ex-native forest sites (Basils, Nunamara, and Tim Shea) between ages 1-2 years, noting that these sites had lower concentrations of mineralisable N than an ex-pasture site (Potters) (Wang *et al.* 1996a). The same five sites were chosen for the current study, their characteristics being summarised in Table 2.1. Measurements of *in situ* NNM

followed on immediately from the Wang *et al.* (1998) study at the Boulder, Basils, Nunamara and Tim Shea sites. The sites had been cleared of trees and shrubs then strip cultivated (about 2.5 m ploughed and 1.5 m unploughed) prior to planting with *E. nitens*. These sites typify a broad range of sites in Tasmania where *E. nitens* plantations are being established. For measurement of *in situ* NNM, soil (0-10 cm) was collected from cultivated and uncultivated regions within randomly distributed control plots (250 m^2 approximate area) that had not received fertiliser during the establishment phase of a N fertiliser experiment covering approximately three ha at each site. All sites had three unfertilised plots, except the Potters site, which had five unfertilised plots. Herbicides were applied to the total area of all plots to control weeds. A few woody perennial weeds grew at the Basils, Boulder, and Nunamara sites, but were not considered to be a significant sink for mineral N uptake. Broad leaf weeds and grasses that required spraying with herbicide grew at the Potters and Tim Shea sites. The control plots at the Tim Shea site were sprayed with 'Roundup' (isopropylamine salt of N-[phosphono-methyl] glycine) in June 1996 and January 1997, the Potters site was sprayed with 'Atrazine' (2-chloro-4-ethyl-6-isopropylamino-s-triazine) in January 1997 and the control plots of the Potters site were sprayed with 'Round up' in April 1997.

2.2.2 Calculation of N fluxes

The same *in situ* soil-core technique used by Wang *et al.* (1998) was used to measure NNM and nitrification in uncultivated soil at all sites for two years. For the same period, net nitrification and leaching were measured within the uncultivated region of all sites except the Potters site. For the first year only, NNM and nitrification were also measured in the cultivated soil at the Tim Shea and Nunamara sites. Rates of

Table 2.1. *Site characteristics. Sites are in order of increasing elevation.*

Characteristic.	Boulder	Nunamara	Tim Shea	Potters	Basils
Previous vegetation	*P radiata*	*E. viminalis*	*E. regnans*	pasture	Euc.- myrtle
Planting date	June 1993	Oct. 1993	Oct. 1993	Oct. 1995	July 1993
Latitude	41°12'	41°21'	42°40'	41°9'	41°19'
Longitude	145°50'	147°15'	146°29'	145°45'	145°39'
Elevation (m)	390	400	420	510	550
Rainfall[A] (mm/year)	1400	1000	1500	1570	1800
Daily soil temperature[B]					
Jan_{min}-Jan_{max} (°C)	15-19	15-19	12-15	13-15	13-15
Jul_{min}-Jul_{max} (°C)	3-7	4-7	3-6	2-6	2-6
Soil type[C]	Ferrosol	Ferrosol	Kurosol	Ferrosol	Ferrosol
Parent Material	Basalt	Basalt	Siltstone	Basalt	Basalt
Surface texture	Clay loam	Clay loam	Clay loam	Clay loam	Clay loam
Bulk density[D] (g/cm^3)	0.77	0.99	0.76	0.60	0.51
α[E]	0	3	0	0	6
pH[F]	5.1	5.8	4.6	4.3	5.0
Total N (%)*	0.27	0.22	0.33	0.65	0.74
Total C (%)*	6.7	3.4	6.1	9.1	13.3

[A] *Approximate long-term mean,* [B] *Approximate average of the daily maximums and minimums (10 cm),*

[C] *Isbell (1996),* [D] *Fraction <5 mm,* [E] *α rock fraction (%),* [F] *1:5 soil:water* **from chapter 3*

NNM and nitrification were calculated as the difference in mineral N between soil collected at the beginning of the incubation (initial soil) and *in situ* incubated covered soil samples. Leaching was calculated as the difference in concentration of mineral N between *in situ* incubated covered soil and *in situ* incubated uncovered soil.

The N fluxes were calculated using the equation:

δN (kg/ha/period) = δN (μg N/g soil/period) $\times$ BD (g soil/cm^3 soil) $\times$ (1-α) $\times$ 10^{-9} kg N/μg N $\times$ 10^8 cm^2 soil/ha $\times$ 10 cm depth of soil.

Where α is the proportion of rocks (0-6%) estimated visually for each plot using the method described by McDonald *et al.* (1990), and BD is bulk density measured on four cores (420 cm^3) from each plot (0-10 cm depth). Soils from each set of bulk density cores were bulked within plots, then separated into coarse and fine fractions using a 5 mm sieve. The soil fractions were dried at 105° C for 24 h and their weights recorded. Only the fraction <5 mm was used to calculate BD, which was 83-98% of the total soil weight. The soil fraction >5 mm was assumed to have negligible contribution to N fluxes (Raison *et al.* 1987; Wang *et al.* 1998). Where N fluxes were indicated as kg N ha^{-1} day^{-1}, they were calculated as the total change in N during the incubation period divided by the number of days. Measurements of annual fluxes of NNM and leaching in each plot were calculated by summing the N fluxes in individual periods over the whole year.

Measurements of N fluxes for this study began on 1st November 1995 at the Basils and Boulder sites, 7th November 1995 at the Nunamara site, 14th November 1995 at the Tim Shea site and 16th January 1996 at the Potters site. There were 16 collection periods at the Potters site, 17 at the Basils and Boulder sites and 18 at the Tim Shea and Nunamara sites. Data were missing from the Nunamara site (two collections) and the Boulder site (one collection suspected of contamination) representing 12% and 6% of collection dates at these two sites, respectively. Missing values, calculated using the average rate of the remaining incubation periods, were very similar and not significantly different from rates measured for the year after or before the missing values.

significantly different from rates measured for the year after or before the missing values.

2.2.3 Soil sampling

Initial soil samples comprised eight cores taken from each plot (0-10 cm depth) using a 50 mm internal diameter stainless steel tube. At the same time, eight PVC tubes (50 mm internal diameter) were pushed into the soil to 10 cm depth in each plot and covered with a PVC cap to prevent leaching during field incubations. In plots where leaching was measured, eight additional PVC tubes per plot were pushed into the soil to 10 cm depth and left uncovered. After 5-8 weeks, incubated soils were collected along with another set of initial samples and the procedure repeated. Plate 2.1 shows covered and uncovered soil cores in uncultivated soil at the Basils site. Collected soil was transported with minimal disturbance to a cool room (4° C) within four hours of collection and processed within three days. Each group of eight like cores (initial, covered or uncovered) were mixed, sieved through a 5 mm mesh and sub-sampled for extraction with 2M KCl.

Plate 2.1 *Covered and uncovered soil cores incubating in uncultivated soil at the Basils site.*

2.2.4 KCl extraction for NH^{4+} and NO^{3-} assay

Subsamples (20 g fresh soil) were placed into a 110 ml screw-cap container with 100 mL 2M KCl, placed on a rotary shaker for one hour, allowed to settle and filtered through Whatman No. 42 filter paper. Extractants were stored frozen (-10° C) and thawed prior to analysis. Ammonium (NH_4^+) was analysed by a modified Berthelot indophenol reaction (Lachat Quikchem method 12-107-06-1-A). Nitrate (NO_3^-) was measured by the cadmium reduction method (Lachat Quikchem method 12-107-04-1-F). Concentrations of NH_4^+ and NO_3^- were reported on an oven dry basis.

2.2.5 Estimation of nominal field capacity (-25 kPa)

Five rubber rings, 10 mm in height and 52 mm in diameter, were placed onto a ceramic disc in a pressure plate chamber. The rubber rings were filled with sieved (< 2 mm), air dried soil. The soil and ceramic disc were soaked in water over night before the pressure plate was sealed and placed under -25 kPa of pressure. After 24 hours the equilibrated soil was removed and oven dry water content determined (weight of water loss after 24 h at 105 °C/weight of dry soil).

2.2.6 Statistical analysis

Means were compared using the least significant difference (LSD) analysis where an analysis of variance (ANOVA) showed a significant difference between means ($P \leq 0.05$). A 2-way ANOVA based on replicates and groups was used for analysis to determine the significance of changes of water contents, mineral N contents, and N fluxes over time within sites. Groups comprised replicate observations of individual means to be compared [e.g. date × N form (NH_4^+-N or NO_3^--N) combinations]. Fluxes for individual periods within sites were compared on the common basis of daily rates. Annual rates of NNM or leaching were compared for sites by 1-way ANOVA. Correlations and regressions were determined by standard statistical methods.

2.3 RESULTS

2.3.1 Soil water

Soil water contents were lowest during December - March and highest during July - November at all sites (Figure 2.1). There were similar trends in the soil water contents

of initial and covered samples at all five sites, however differences between the initial and covered soils were inconsistent. At the Basils and the Potters sites, soil water contents were similar to, or greater than, nominal field capacity (-25 kPa) for the 2-year study period. At the Boulder and Nunamara sites, water contents dropped below –25 kPa during December to March each year, and the same occurred at the Tim Shea site during January to March each year. During the remaining months of higher rainfall (Appendix) soil water contents within sites were roughly constant at what is likely to be actual field capacity. Least significant differences in water content of soil between sampling dates within sites were significant and small (3.7-8.3%), and hence are not shown in Figure 2.1.

2.3.2 Net N mineralisation

Rates of NNM were highly variable, hence no significant differences between years in annual rates of NNM were measured at any site and annual rates were not significantly different to those measured by Wang *et al.* (1998) for the Basils, Boulder, Tim Shea, and Nunamara sites in the year prior to this study (Table 2.2). There were no significant differences in annual rates of NNM between cultivated and uncultivated soils at the Tim Shea and Nunamara sites during the first year of this study (Table 2.3). Therefore measurements were made in uncultivated zones only during the following year.

Average rates of NNM for individual sampling periods ranged from -0.3 kg N ha^{-1} day^{-1} at the Potters site during July-October 1996 to 1.8 kg N ha^{-1} day^{-1} at the same site during January-March 1996 (Figure 2.2), resulting in annual rates that covered a ten-fold range: from 13-188 kg N ha^{-1} $year^{-1}$ (Table 2.2). Significant differences between sampling dates for NNM occurred at the Basils and Potters sites (Figure 2.2). At the Potters site, rates of NNM were highest during summer (December-March) and lowest during the winter (June-September). In contrast, at the Basils site, differences in NNM were not consistent with a seasonal pattern.

Figure 2.1. *Gravimetric water content at the Boulder, Nunamara, Tim Shea, Potters, and Basils sites from December 1995 to January 1998. Horizontal lines represent water content at -25 kPa.*

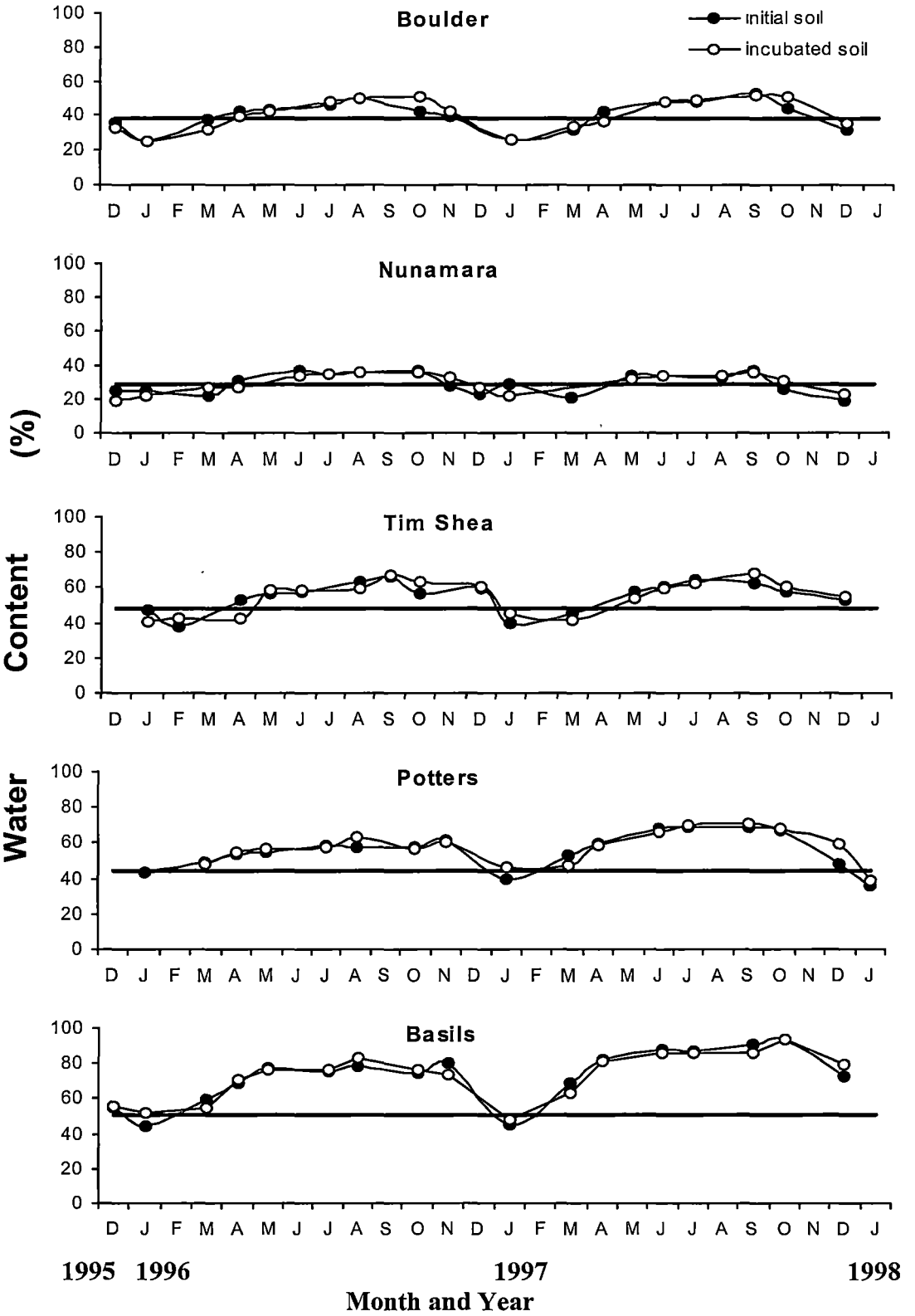


Table 2.2. *Annual N fluxes and pools of mineral N (kg ha^{-1}) in uncultivated surface soils (0-10 cm) of five Tasmanian* E. nitens *plantations. For mineralisation and mineral N data, transformations were inadequate to satisfy homogeneity of variance constraints for an ANOVA. Therefore, as indicated by pair-wise comparisons for untransformed data using t-tests for means of unequal variance, homogeneous groups of years within sites (P < 0 05) are accompanied by the same upper-case letter, and homogeneous groups of sites within years are accompanied by the same lower-case letter Because there were no significant differences between years for mineralisation at any site, sites meaned across years were also compared For other datasets, homogeneous groups of means across sites and years are accompanied by the same letter as indicated by a Duncans multiple range test using log-transformed (nitrification) or untransformed (leaching and uptake) data Parenthesis contain standard deviations*

	Site				
	Boulder	Nunamara	Tim Shea	Basils Potters	
Mineralisation					
94/95[1]	18 (37) A a	23 (47) A a	54 (43) A a	91 (47) A a	n.d.
95/96	24 (7) A a	23 (7) A ab	12 (14) A a	70 (22) A b	188 (91) A c
96/97	13 (1) A a	20 (9) A a	23 (6) A a	77 (33) A ab	175 (84) A b
Mean	18 a	22 a	30 a	79 b	182 c
Nitrification					
94/95[1]	22 (16) ef	39 (33) cde	51 (30) cd	72 (30) bc	n.d.
95/96	18 (3) ef	20 (9) def	2 (1) g	52 (20) b	191 (75) a
96/97	4 (0) g	12 (6) f	2 (1) g	61 (22) b	165 (70) ab
Leaching					
94/95[1]	36 (3) ab	n.d.	n.d.	61 (21) a	n.d.
95/96	22 (12) bc	n.d.	n.d	50 (22) ab	n.d.
96/97	-5 (7) c	n.d.	n.d.	46 (25) ab	n d.
Uptake					
95/96	13 (14) b	n.d.	n.d	16 (7) b	n.d.
96/97	20 (8) ab	n.d.	n.d.	36 (7) a	n.d.
Mineral N					
October 94[1]	17 (13) AB a	16 (8) AB a	13 (0) A a	11 (1) A a	n.d.
October 95[1]	10 (7) A a	6 (1) A a	6 (3) AB a	5 (1) B a	n.d.
October 96	2 (1) B a	4 (1) AB ab	4 (1) B a	6 (1) B bc	8 (2) A c
October 97	4 (1) B ab	3 (1) B a	2 (2) B a	6 (5) AB ac	6 (3) A bc

[1] *From Wang et al (1998)*

n.d. *indicates not determined.*

Table 2.3. *Annual net N mineralisation and nitrification (kg ha^{-1}) in cultivated and uncultivated soil at the Tim Shea and Nunamara sites for the period November 1995 - November 1996. Difference in N fluxes between cultivated and uncultivated soil were not significantly different (P < 0.05).*

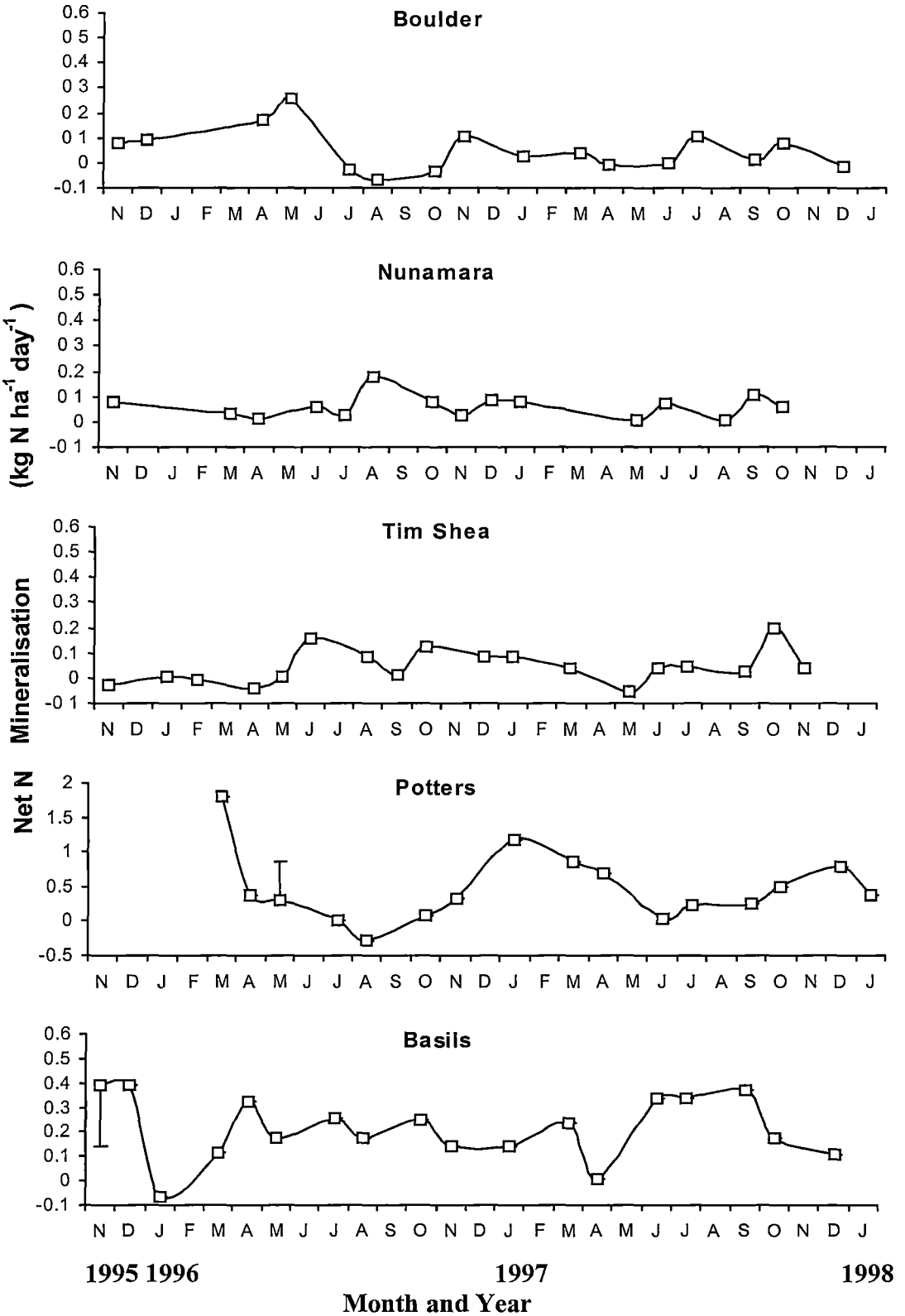
	Site	
	Nunamara	Tim Shea
N mineralisation		
Cultivated	27 (11)	13 (14)
Uncultivated	23 (7)	12 (14)
Nitrification		
Cultivated	22 (6)	-3 (0.8)
Uncultivated	20 (9)	2 (0.7)

Parenthesis denote standard deviation (n=3)

2.3.3 Net nitrification

Average estimated rates of net nitrification for individual sampling periods ranged from an apparent -0.26 kg N ha^{-1} day^{-1} at the Potters site during July-October 1996 to 1.8 kg N ha^{-1} day^{-1} at the same site during January-March 1996, resulting in annual rates of 2-191 kg N ha^{-1} year^{-1} (Table 2.2). Significant differences in net nitrification rates occurred at all sites between sampling dates except at the Nunamara site, but these differences were not consistent with a seasonal pattern. At all sites rates of nitrification were highly variable and not significantly different to rates of NNM. Further evidence of variability in nitrification data was provided at the Basils and Potters sites there was a period of significant ($P < 0.05$) apparent negative nitrification.

Figure 2.2. *Net N Mineralisation (kg N ha^{-1} day^{-1} in situ; 0-10 cm depth) from November 1995 to January 1998 at the Boulder, Nunamara, Tim Shea, Potters, and Basils sites.*



For sites with significant differences between sampling dates, bars denote LSD
Note the different scale of the Potters site y-axis.

2.3.4 Leaching

At the Boulder site, considerable leaching of N from the top 10 cm was observed during the Wang *et al.* (1998) study and during the first year of this study, which included both NH_4^+ and NO_3^- (Figure 2.3). However, during the last year, leaching of both forms of mineral N was minimal. At the Basils site considerable leaching was recorded in all three years, and it was dominated by NO_3^-. Average rates of leaching for individual periods were -0.30 to 0.16 kg N ha^{-1} day^{-1} and 0.00 to 0.44 kg N ha^{-1} day^{-1} for the Boulder and Basils sites respectively (Figure 2.3). There were no significant differences in rates of N leaching between sample periods.

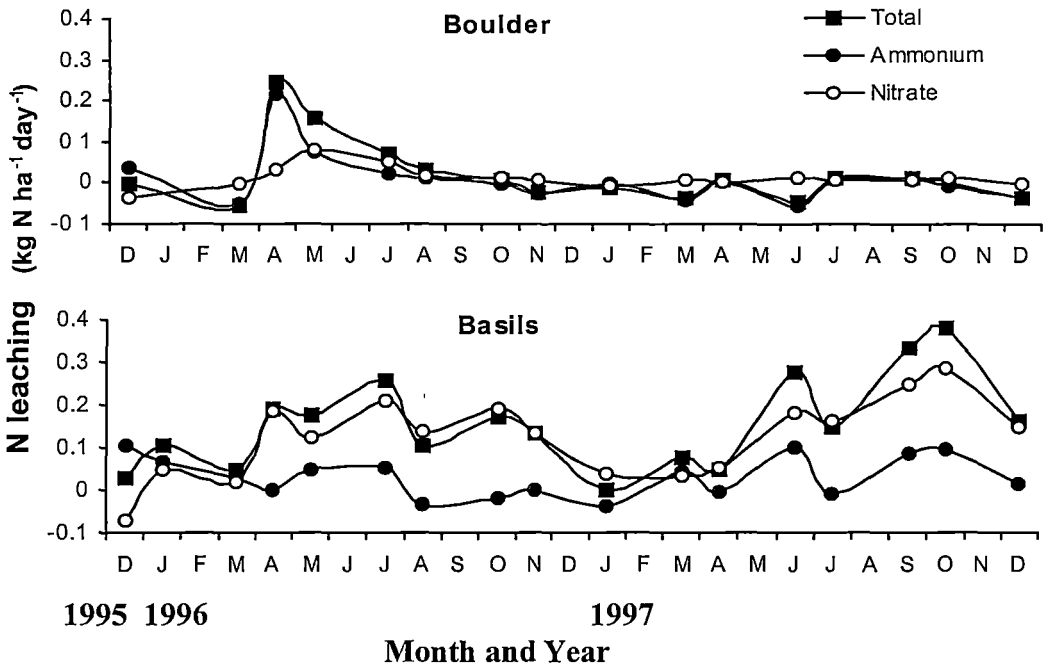
The Basils site showed higher average rates of leaching than the Boulder site in all years of measurement but differences were significant only in the last year of measurement (Table 2.2). Lowest rates of leaching at the Basils site occurred during summer (December-February) (Figure 2.3). Rates of N leaching and NNM were not significantly different ($P<0.05$, $n=3$ for all sites except for the Potters site where $n=5$). Hence, leaching (within the errors of measurement), could have accounted for all N mineralised to 10 cm depth during the two years of measurement.

2.3.5 Mineral N

Amounts of mineral N ($NH_4^+ + NO_3^-$) extracted from soil for individual sampling dates ranged from 0.7 kg N ha^{-1} at the Tim Shea site in September 1997 to 93 kg N ha^{-1} at the Potters site in March 1997 (Figure 2.4). Amounts of mineral N extracted from soil in October 1996 and October 1997 ranged from 3 kg N ha^{-1} at the Nunamara site in 1997 to 8 kg N ha^{-1} at the Potters site in 1996 (Table 2.2). Significant differences between mineral N extracted on individual sampling periods occurred at all sites, but these differences were consistent with a seasonal pattern only at the Potters sites (Figure 2.4).

During the study period, NO_3^- formed 0-64%, 13-42%, 4-49%, 31-96%, 23-80% the mineral N present at the Boulder, Nunamara, Tim Shea, Potters, and Basils sites, respectively. A significant trend of decreasing amounts of mineral N with time was observed at the Boulder, Nunamara, and Basils sites (Figure 2.4).

Figure 2.3. *Leaching (kg N ha^{-1} day^{-1} in situ; 0-10 cm depth) from December 1995 to December 1997 at the Boulder and Basils sites.*

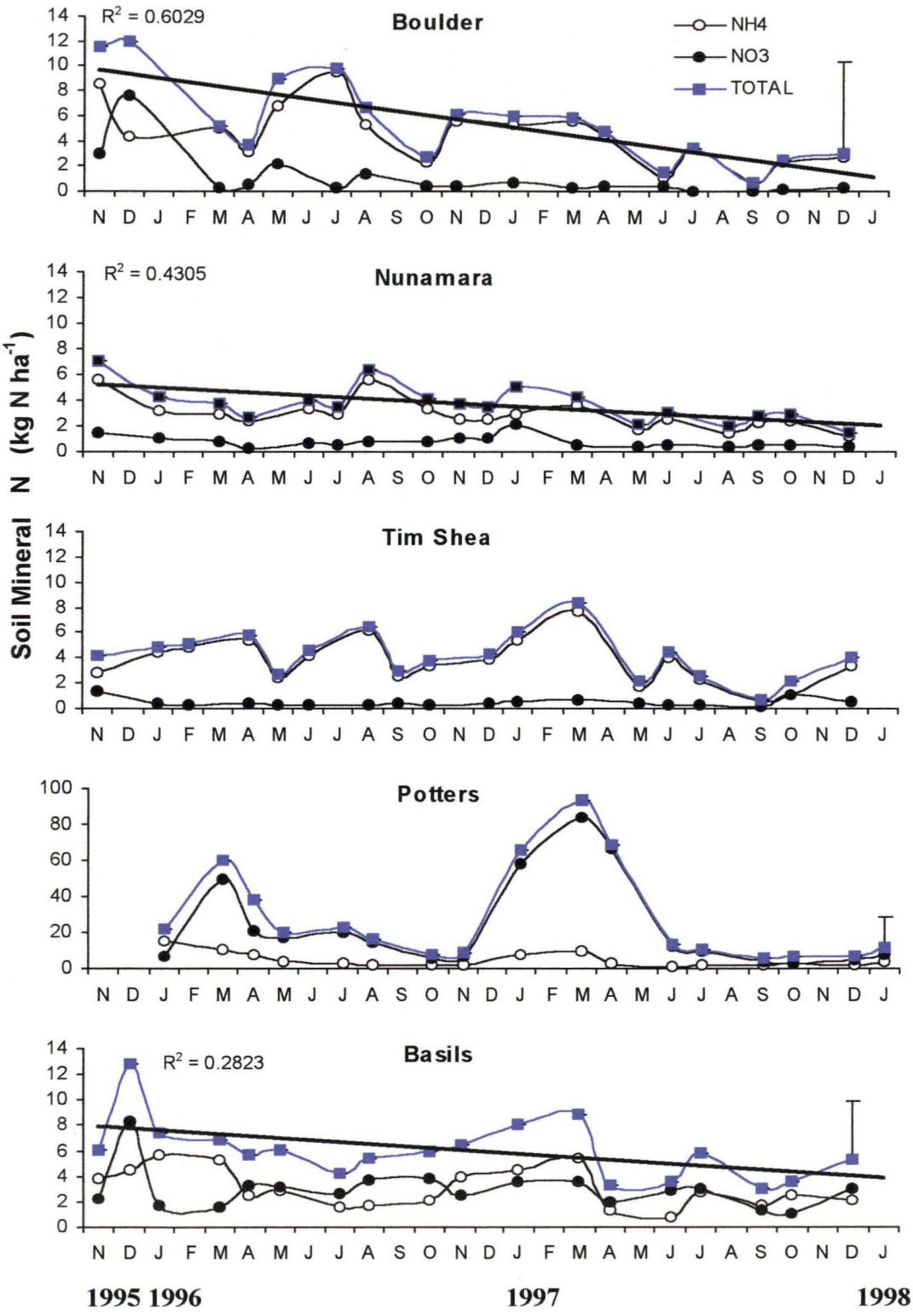


Differences between sample periods were not significant.

2.3.6 N uptake

Observed rates of N uptake ranged from -0.12 to 0.22 kg N ha^{-1} day^{-1} (data not presented) which resulted in N uptake estimates of 13 to 36 kg N ha^{-1} year^{-1}. Differences in estimated rates of N uptake between sites and between years within sites were not significant. However rates of N uptake at the Basils site ranked above the Boulder site for both years of measurement (Table 2.2). Rates of NNM ranked above N uptake at both sites for both years of measurement and were significantly greater at the Basils site in the second year.

Figure 2.4. *Mineral N content (kg N ha^{-1}; 0-10 cm) from November 1995 to January 1998 at the Boulder, Nunamara, Tim Shea, Basils, and Potters sites. For total (NH_4^+ + NO_3^-), bars are LSD (P = 0.05), regression lines and r^2 values are shown where significant (P < 0.05) trends in decreasing mineral N occurred.*



Note the different scale of the Potters site y-axis

2.4 DISCUSSION

Rates of NNM (0-10 cm) covered a wide range (13-188 kg N ha^{-1} $year^{-1}$), which despite the generally shallower sampling depth in the present study matched the whole of the range found in studies of other Australian plantation forest soils. For example, from 16 kg N ha^{-1} $year^{-1}$ (0-15 cm) in *P. radiata* from South Australia (Carlyle *et al.* 1998) to 113 kg N ha^{-1} $year^{-1}$ (0-20 cm) in *E. globulus* plantations of south-western Australia (O'Connell and Rance 1999). There was no significant decrease in rates of NNM for surface soils of plantations during ages 1-4 years which was in contrast to several other studies. For example, in South Australia, Smethurst and Nambiar (1990a) found a decrease in rates of NNM from 50-70 kg ha^{-1} during the first year to 20-30 kg ha^{-1} during the third year after planting of *P. radiata*. Raison *et al.* (1992) found a decrease in rates of NNM from 38 to 7 kg N ha^{-1} $year^{-1}$ during a four-year period in a *P. radiata* plantation. Although there was some evidence of decreasing rates of NNM during the measurement period (Table 2.2) and higher rates due to cultivation (Table 2.3) effects were insignificant and indicative of high variability.

Annual rates of NNM at the Potters ex-pasture site were significantly higher than at the other sites, which were ex-mixed forest and ex-pine. Other authors have observed high rates of N mineralisation in pasture soils. For example, rates of gross N mineralisation were increased from -0.01 to 2.5 kg N ha^{-1} day^{-1} by converting from wheat to pasture (Murphey *et al.* 1998) and NNM was shown to increase in soil with the age of the pasture (Speir *et al.* 1982). High rates of NNM at the Potters ex-pasture site may be due to a higher quality or quantity of organic matter resulting from previous pasture at this site (Wang *et al.* 1996). The beneficial effects of pasture on OM quality may subside with time from conversion to eucalypt plantations. For example Aggangan *et al.* (1998) show low rates of NNM during aerobic incubation in native forest soil, high rates in pasture soil and intermediate rates in *Eucalypt* plantation soil of ex-pasture and ex-native forest. The Potters ex-pasture site had greater than double the rates of NNM (0-10 cm depth) than the Basils ex-native forest site. This was not reflected in values of total N and total C which were higher at the Basils site, indicating that the Potters ex-pasture site had a better quality of OM. Organic matter quality is examined further in chapter three. Annual rates of NNM at

the Tim Shea and Nunamara ex-mixed forest sites were not always ranked intermediate between the Potters ex-pasture and the Boulder ex-pine sites, as was anticipated by Wang *et al.* (1996) (Table 2.2), suggesting any possible deleterious effects of *P. radiata* on organic matter quality are not as great as the positive effects of pasture on organic matter.

Because soil water content at all sites was likely to have been at or close to field capacity for most of the year, it is unlikely that low water content was a significant limitation to rates of NNM. It is also unlikely that high water contents inhibited NNM because all soils are well drained and probably had high partial pressures of oxygen as a result of low temperatures. Soil (0-10 cm) temperature differences between sites were small <5 °C (Table 2.1, Appendix) and the two coldest and wettest sites (Basils and Potters) also had the highest rates of NNM. Therefore, differences in soil temperature and moisture content could not account for differences in NNM rates across the sites. Wang *et al.* (1988) came to the same conclusion for four of these study sites for the period October 1994 to October 1995, where differences in rates of NNM could be attributed to differences in mineralisable substrate as indicated by anaerobically mineralisable N and total N. Only at the Potters site was there a strong seasonal pattern of NNM with summer highs and winter lows. From January to March temperatures were high and soil water content was low. Generally within the experienced temperature and water contents, we expect NNM to decrease as soil water content decreases and NNM to increase as temperature increases. Hence, seasonal rates of NNM at the Potters site are probably temperature-driven. At the other four sites, seasonal changes in temperature or water content were not associated with a seasonal pattern in NNM; this may be attributed to the high variability in NNM measurements.

Rates of NNM were not significantly different to rates of nitrification, resulting in high proportions of mineral N as NO_3^- observed at all sites. While some studies show Australian forest soils do not have high proportions of NO_3^- (Adams and Attiwill 1986 and 1991; Polglase *et al.* 1992; Raison *et al.* 1987; Smethurst and Nambiar 1990a), others do. For example, Ellis *et al.* (1982) found NO_3^- was 0.2-84% of mineral N produced during aerobic incubation of a forest soil from southern Tasmania. Ellis and

Graley (1983) and Ellis and Pennington (1989) found NO_3^- formed 0-21% and 0-60% of mineral N in several Tasmanian soils respectively. High nitrification rates at the Tim Shea, Nunamara, Boulder, and Basils sites during this study (November 1995 to November 1990), were similar to those of Wang *et al.* (1988) during October 1994 to October 1995 (Table 2.2). These results indicate that conditions at all the study sites were favorable for nitrification. Nitrate made up on average 13, 19, 21, 49, and 82% of mineral N extracted at the Tim Shea, Nunamara, Boulder, Basils, and Potters sites, respectively. Nitrate was present in all initial samples except at the Boulder site in September 1997, with high proportions of NO_3^- at all times of the year at the Potters (31-96%) and Basils (23-80%) sites. At no site was there a significant net change in mineral N content between October 1996 and October 1997, however there were differences for other sampling dates and the mineral N content at the Boulder, Nunamara, and Basils sites generally decreased with time (Figure 2.4). This decrease in soil mineral N was not associated with significantly lower NNM rates, and, therefore, it was more likely to be due to increasing rates of N uptake by the plantations.

Leaching at the Basils site during November 1994 to November 1995 (Wang *et al.* 1998), and in this study during November 1995 to November 1997, accounted for all N mineralised (0-10 cm depth), within the errors of measurement, and leaching was dominated by NO_3^- (Table 2.2; Figure 2.). Hence large annual rates of leaching at the Basils site reflect the high rates of mineralisation, nitrification, and rainfall at this site. Leaching at the Boulder site during the second year was significantly lower than at the Basils site, reflecting lower rates of NNM, nitrification, and rainfall at the Boulder site. Leaching at the Boulder site included periods with apparent negative rates and high amounts and proportions of NH_4^+ on two occasions. Apparent negative rates of leaching and nitrification may be artifacts of the method resulting from sampling errors and differences in soil water, temperature, and N fluxes that can occur between uncovered and covered soil and in cored and un-cored soil (Raison *et al.* 1987). These effects may have resulted in differences in rates of denitrification and immobilisation between these soils, further contributing to apparent negative rates of leaching. Apparent negative rates of leaching were very common during 1996/97, when concentrations of mineral N were very low and these differences more likely to result

in apparent negative rates. Results reported in chapter three show the Boulder site to have a C:N ratio of 25. When the C:N ratio of soil is 20 or greater, immobilisation of soil N is likely to occur, which can cause decreases in soil NH_4^+ and NO_3^- levels (Tisdale *et al.* 1993; Leeper 1982), potentially explaining the apparent negative rates of leaching.

Although large amounts of NO_3^- are leached beyond the 0-10 cm depth, much of this NO_3^- may be intercepted by deep penetrating roots (eg, Tasmanian *E. nitens* root system to 80 cm depth at 34 months Misra *et al.* 1998a) as it moves down the profile or held on the subsoil anion exchange, common to ferrosols (Black and Waring 1976). Hence, although large leaching N losses occur in the 0-10 cm depth, losses from the soil profile explored by the root system may be small.

In summary this study has confirmed several trends previously established by Wang *et al.* (1996a, 1996b, 1998):

- High *in situ* rates of nitrification and leaching occur within the study sites.
- Few seasonal patterns are seen in NNM and nitrification.
- The ex-pasture Potters site has much higher *in situ* rates of NNM than the other sites examined.

There are also several important new findings:

- Rates of *in situ* NNM in cultivated and uncultivated soil were not significantly different.
- No significant decrease in rates of *in situ* NNM were observed between plantation ages 1-4 years.
- *In situ* NNM at the Potters ex-pasture site was very high and approximately double the rates of *in situ* NNM previously measured in Tasmania.

3. INDICIES OF NITROGEN MINERALISATION

3.1 INTRODUCTION

The *in situ* soil-core technique is favoured for measuring field rates of NNM, but this method is labour- and time-intensive and not suited to broad-scale management practices. A simpler soil analysis correlated to rates of NNM in the field would aid in selection and judicious fertilisation of plantation sites.

Of total soil N only a small portion is mineralised (Bremner 1965). It is this portion of labile soil N that soil analyses attempt to extract or correlate to, whether the procedure is via physical separation, chemical extraction, which is usually via acids, bases or salts, or by microbial incubation, which utilize the native microbiota responsible for mineralisation and are either aerobic or anaerobic (Bremner 1965, Keeney 1982). Generally OM compounds increase in recalcitrance from very labile soluble monomolecular substances (sugars, organic acids, amino acids, lipids, nucleotides) to soluble cell components (organic acids, lipids, nucleotides, sugars) to peptides and proteins to nucleic acids to polysaccharides, cellulose and lignin (Singer and Donald 1996).

Extensive literature reviews covering the various indicies of N availability and their usefulness have been published by Bremner (1965), Dahnke and Vasey (1977), Keeney (1982), Binkley and Hart (1989), and Rice and Havlin (1994). Indicies examined in this chapter fall into two categories: biological mineralisation (incubation) and chemical extraction.

Aerobic incubation methods measure the increase of NH_4^+ and NO_3^- in soil, usually at a constant temperature and water content. Pre-treatment disturbance can affect rates of NNM (Bremner 1965; Binkley and Hart 1989), resulting in an initial flush of N and measured rates that are dependent on the length of incubation (Binkley and Hart 1989). There are a variety of incubation conditions in use, but commonly temperatures are in the range 20-25 °C and the duration is 10-30 days. However, periods in the vicinity of 60 days are not uncommon, for example 56 days (Carlyle

et al. 1990) and 68 days (Connell *et al.* 1995). Warmer temperatures also have been used, for example 30 °C (Oien and Selmer 1980; Keeney and Bremner 1966a; Gianello and Bremner 1986b), and 35 °C (Stanford and Smith 1972). Anaerobic methods involve measuring the increase in NH_4^+ only during incubation of flooded soil in a sealed container (e.g. Wang *et al.* 1998). Water content, aeration, and NO_3^- do not require monitoring because nitrification does not occur and therefore denitrification rates are of no concern. Keeney and Bremner (1966a) and Powers (1980) found that during the more common anaerobic incubation conditions of 40 °C for seven days (e.g. Thicke *et al.* 1993; Hart and Binkley 1985; Myrold 1987; Stanford and Smith 1972; Gianello and Bremner 1986b; Hart *et al.* 1986) similar amounts of nitrogen were mineralised to those found after for 14 day incubations at 30°C (e.g. Waring and Bremner 1964; Adams and Attiwill 1986b). Anaerobic procedures generally mineralise more N than aerobic procedures and have a shorter incubation period (Thicke *et al.* 1993; Adams and Attiwill 1986b; Hart and Binkley 1985; Binkley *et al.* 1992). Nitrogen mineralised during anaerobic incubation has been well correlated with microbial biomass, suggesting that N mineralised during anaerobic incubations comes mainly from the soil microbial biomass killed during the incubation (Myrold 1987; Azam *et al.* 1988; Adams and Attiwill 1986b). Powers (1980) found anaerobically mineralisable N decreased exponentially with soil depth paralleling the usual distribution of organic N.

Anaerobically mineralisable N has indicated N sufficiency in field crops. For example, anaerobically mineralisable N correlated with maize yield, leaf %N, and N uptake (Robinson 1967; Cornforth and Walmsley 1971), with sorghum yield, and N uptake (Ryan *et al.* 1971), with rye grass yield, N uptake, and response to N fertiliser (Gasser and Kalembasa 1976; Osborne and Storrier 1976). Within forest soils, anaerobically mineralisable N correlated with response of douglas fir to added N (Shumway and Atkinson (1977, 1978). Hence, anaerobically mineralisable N may correlate with NNM in eucalypt forest soils of Tasmania. The anaerobic procedures used in this assay are shorter and simpler than aerobic procedures, making them more suitable for routine use.

Non-incubation methods that involve chemical extraction are attractive because they are more rapid, convenient, and precise than biological methods and less affected by preliminary soil sampling, handling, and storage (Gianello and Bremner 1986b; Selmer-Olsen *et al.* 1981; Oien and Selmer-Olsen 1980; Hart and Binkley 1985). Among chemical methods, hot KCl extractable N has potential for providing an estimate of mineralisable N (Campbell *et al.* 1994) because it is correlated with anaerobically mineralisable N ($r^2 = 0.95$ Gianello and Bremner 1986b; $r^2 = 0.81$ Aiman 1992; $r^2 = 0.98$ Selmer-Olsen *et al.* 1981), aerobically mineralisable N ($r^2 = 0.93$ Gianello and Bremner 1986b; $r^2 = 0.92$ Oien and Selmer-Olsen 1980), as well as crop growth and N uptake (Whitehead 1981; McTaggart and Smith 1993; Selmer-Olsen *et al.* 1981; Aiman 1992). The hot KCl method measures the increase in NH_4^+ during heating of soil in KCl for periods of 1-24 hours at 80-105 °C. However, it is noted that extraction period (McTaggart and Smith 1993; Oien and Selmer-Olsen 1980) and temperature (Oien and Selmer-Olsen 1980) are positively correlated with the total N extracted.

Nitrogen uptake and growth of plants in greenhouse experiments and biological and chemical analyses of N availability have been positively correlated with concentrations of soil total N and total C (Keeney 1982; Dahnke and Vasey 1977) but there are many irregularities (Danke and Vasey 1977). Although total N, total C, and C:N ratios are not universal indicators of N mineralisation (Richards *et al.* 1985), N mineralisation has been positively correlated with total N in several Australian forest soils when soils were grouped into primary profile form ($r = 0.82$) (Connell *et al.* 1995) or into strongly ($r^2 = 0.59$) and weakly ($r^2 = 0.65$) nitrifying soils (Carlyle *et al.* 1990). Positive correlations with total N and anaerobically mineralisable N have been reported, for example by Gianello and Bremner (1986a) ($r^2 = 0.79$), and Fox and Piedielek (1984) ($r^2 = 0.79$). Usually small fractions (<5%) of the total soil N and total soil C are mineralised, hence total N and total C are expected to be temporally stable.

Wang *et al.* (1998) ranked two of the present study soils (Basils and Boulder) in order of *in situ* NNM using total N, anaerobically mineralisable N, and hot KCl extractable N. Hence these soil analyses are likely indices of NNM for Tasmanian forest soils.

Pinus radiata stands have been reported to accumulate additional N on the addition of P fertiliser (Waring 1969; Nielson *et al.* 1984; Turner and Lambert 1986), show increased growth and foliar N concentrations (Crane 1981; Farrell 1990), and show a positive growth interaction with N fertilisation (Cromer *et al.* 1975; Waring 1981). Positive growth responses to N fertiliser were more common with high soil extractable P (Radwan and Shumway 1983). Application of superphosphate increased NNM in a dry sclerophyll *Eucalypt* forest from 20.7 kg N ha^{-1} $year^{-1}$ to 28.3 kg N ha^{-1} $year^{-1}$ (0-20 cm) (Falkiner *et al.* 1993). Phosphorous may have a positive effect on soil microbiota, increasing their activity (Falkiner *et al.* 1993). Hence Tasmanian forest sites of high total P may have higher rates of NNM. Large amounts of P can be found as organic P (Kelly *et al.* 1983) and as P in association with soil minerals (P sorption) (Mendham 1998), hence total P is expected to be temporally stable.

The process of NNM releases NH_4^+ from soil organic nitrogen, which is readily converted to NO_3^- via nitrification. It is possible that soils of high NNM will also have high concentrations of NH_4^+ and NO_3^-. For example, in soil after burning of a north American pine-hardwood forest, soil solution concentrations of NH_4^+ and NO_3^- increased with increases in NNM rates, proportions of NO_3^- increased with increases in *in situ* nitrification rates, and aerobically mineralisable N (Knoepp and Swank 1995). NNM, uptake, and soil solution N were higher in soil from a maple forest than a less productive pine forest (Hill and Shackleton 1989). Soil solution N concentrations were also higher under black locust forests than under pine-mixed hardwoods of lower NNM rates (Montagnini *et al.* 1986). Hingston and Jones (1985) found an increase in soil solution N after N fertilisation of *E. marginata* forest in Western Australia.

Of concern however, is the possibility of seasonal fluctuations in concentrations of mineralisable N (Bremner 1965). For example, Adams and Attiwill (1986) and Powers (1980) found higher concentrations of anaerobically mineralisable N when soil was collected during summer. This may be explained by changes in microbial biomass (Bonde *et al.* 1988) as affected by environmental factors such as soil moisture (Cabrera *et al.* 1994). Hence, there may be preferred periods of sampling if a soil index has considerable temporal variation.

Another concern is that contributions of NNM from the soil profile below the sampled depth (0-10 cm) may represent a significant proportion of the total-profile NNM. Misra *et al.* (1998a) show that fine (< 1 mm) and medium (1-3 mm) roots of a Tasmanian *E. nitens* plantation grew below 80 cm at age 34 months. Hence N availability below 10 cm may form a significant source of mineral N for *E. nitens* plantations. Measurement of *in situ* rates of NNM in subsoils is labour- and time-demanding, hence, was not measured in this study. Soil analyses have the potential to estimate the contribution of subsoil NNM.

Objectives of the research reported in this chapter were: (1) To determine the temporal stability of cold KCl extractable N, soil solution N, anaerobically mineralisable N, hot KCl extractable N, total N, total C, and total P and compare these values with *in situ* rates of NNM. (2) To provide an estimate of subsoil NNM using soil analyses.

3.2 MATERIALS AND METHODS

3.2.1 Site description and sampling

The same five sites described in chapter 2 were sampled in this study. Soil was sampled in the uncultivated regions of the Boulder, Nunamara, Tim Shea, Potters, and Basils sites as for initial soil described in 2.2.3. For all sites n=3, except for the Potters site where n=5.

3.2.2 Analytical methods

Analytical procedures conducted fresh sieved soil were initiated within seven days from sieving. Procedures were conducted in order of cold KCl extraction, anaerobically mineralisable N and soil solution N. For procedures requiring air-dried soil, this soil was sub-sampled within three days of sieving.

Cold KCl extractable N

Cold KCl extractable N is that mineral N (NH_4^+ and NO_3^-) extracted from fresh sieved initial soils using 2M KCl, as described in 2.2.4.

Soil solution N

Soil solution NH_4^+ and NO_3^- were determined for fresh sieved initial (see section 2.2.2) soil in a similar way to Smethurst *et al.* (1997). A sub-sample of 240 g moist soil was made into a paste with the addition of water (50-100 mL, depending on the water content of the initial soil) and mixed for one minute with a spatula until a uniform paste. The mixture was allowed to equilibrate for one hour before being transferred to centrifuge tubes for centrifuging at 2500 rpm for 40 minutes. The supernatant was filtered through cellulose acetate 0.45 μm membrane. Solutions were frozen (-10 °C) until analysis using Lachat Quikchem method 12-107-06-1-A for NH_4^+ and method 12-107-04-1-F for NO_3^-, except water was used as the carrier instead of KCl. Ammonium concentrations in the undiluted soil solution were estimated using the paste method described in Smethurst *et al.* (1997). Table 3.1 shows fitted parameters A and B and Kd values used for calculations of initial soil NH_4^+ concentrations from paste N concentrations.

Anaerobic incubation

Anaerobically mineralisable N was determined for fresh sieved initial (see section 2.2.2) soils using the same method as Wang *et al.* (1998), based on Keeney (1982). A 20 g subsample of fresh initial soil was incubated at 40 °C for seven days in an air tight 100 mL jar with 50 mL water. At the end of the incubation 50 mL of 4 M KCl was added to each jar which was then shaken for one hour. Extracts were filtered through Whatman No. 42 filter papers, stored frozen at -10 °C, then thawed and analysed colorimetrically with a flow injection analyser using Lachat Quikchem Method 12-107-06-1-A for NH_4^+, and Method 12-107-04-1-F for NO_3^-.

Table 3.1. *Fitted parameters A and B, and Kd values used for calculations of initial soil NH_4^+ concentrations using the paste method (Smethurst et al. 1997).*

	Fitted parameters		
Site	**A**	**B**	**Kd values**
Boulder	0.009	3.3396	4.2-400
Nunamara	0.0191 [Z]	3.93138 [Z]	2.7-145
Tim Shea	0.01871 [Z]	8.12458 [Z]	1.4-43
Potters	0.009	3.3396	4.9-43
Basils	0.009	3.3396	1.4-43
Rabbits	0.009	3.3396	12.1-12.7
Old Park	0.009	3.3396	9.0-12.1
Blue Gum	0.001 [X]	1.779 [X]	3.1-3.4
Tabot	0.009	3.3396	13.7-15.0
Wattle	0.009	3.3396	9.9-13.7
Chromeys	0.009	3.3396	4.1-5.3
Wages	0.009	3.3396	14.3-19.9
Basalt	0.009	3.3396	4.9-9.8

[Z] *Data from Smethurst et al. in press.*

[X] *Data for a sand from Umea, Sweden, from Smethurst et al. (1999).*

Remaining fitted parameters were supplied by the Cooperative Research Centre for Sustainable Production Forestry, Hobart, Australia.

Hot KCl extractable N

Hot KCl extractable N was determined for air dried sieved initial (see section 2.2.2) soils. A 3 g subsample of air dried initial soil was heated to 95°C with 20 mL 2 M KCl in 75 mL digestion tubes in an aluminium block digester for 17 hours. After cooling to room temperature, samples were diluted to 100 mL with 2 M KCl. The mixtures were shaken for 15 seconds and the extract filtered through Whatman No. 42 papers. The extracts were frozen (-10 °C) until analysis for ammonia using Lachat Quikchem Method 12-107-06-1-A.

Total N

Total N was determined on air dried sieved initial (see section 2.2.2) soils from soil collections six months apart. An acid digestion technique similar to that described by Rayment and Higginson (1992) method 7A2 was used. A subsample (0.2-0.5 g) of finely ground (< 0.5 mm) air-dry soil was placed into a 75 mL digestion tube. Water was added drop-wise to the soil until damp and left for one hour when 8 mL of sulphuric-salicylic acid (33.3 g salicylic acid in 1 L concentrated sulphuric acid) was added to each tube and mixed before covering and allowing to stand overnight. Sodium thiosulphate (0.5 g) was added to the tubes, which were then heated to 110 °C for ten minutes or until frothing ceased, after which they were allowed to cool. Once cool, 2.2 g catalyst (10:1 w:w anhydrous sodium sulphate: anhydrous copper sulphate) was added before reheating to 360 °C for two hours after the solution cleared and the soil particles appeared white. After cooling for 15 minutes the solutions were made up to 50 mL with water and analysed colorimetrically with a flow injection analyser using a modified Lachat Method 10-107-06-2E. The following modifications were made to this method:

1. The carrier/dilution acid concentrations were based on 96% acid recovery after digestion.
2. Anhydrous dichloroisocyanuric acid-sodium salt (Domestos) replaced 5.25% NaCl
3. 80g/L NaCl was used to remove mercury interference in the alkaline chemistry.
4. Acid/K_2SO_4 solution was used as carrier.
5. Sample volume was able to be varied without change to pH cell effluent.
6. The heater coil was lengthened from 650 cm to 825 cm.
7. Tartate/phosphate/caustic buffer was used, avoiding the problem of crystallisation that occurred using EDTA/NaOH buffer.

Reference soils of known total N were included with each run to provide an estimate of N recovery.

Total P

Total P was calculated using the same method and soil samples as total N, except during the heating to 360 °C for two hours, glass tear drop stoppers were placed on each digestion tube to prevent losses of P. Solutions were analysed colorimetrically with a flow injection analyser using Lachat Method 10-115-01-1D with the following modifications:

1. The carrier/dilution acid concentrations were based on 96% acid recovery after digestion.
2. The carrier was the same as described for total N, enabling analysis of total N and total P together.
3. Chemistry manifold changes:
    a. Reagent pump tubes were increased in volume and molybdate concentrations were adjusted to achieve an optimum (H+)/(molybdate) ratio of 70 and cell effluent pH 0.6 - 0.9 providing good precision.
    b. The heater coil was lengthened from 650 cm to 825 cm.

These modifications allowed sample volume to be varied without change to pH cell effluent. Reference soils of known total P were included with each run to provide an estimate of P recovery.

Total Carbon

Total carbon was calculated from loss-on-ignition data, for fresh sieved initial (see section 2.2.2) soils, using regression relationships determined by Wang *et al.* (1996b) for Tasmanian soils. Loss on ignition was determined by measuring weight loss of 105 °C oven-dried soil samples following heating at 375 °C for 17 hours.

3.2.3 Sampling of Subsoils

Samples were taken from soil pits, one within each of three control plots, at the Potters, Basils, and Boulder sites. Four 50 mm internal diameter PVC cores were used to sample soil from 0-10 cm depths and horizontally into the vertical wall of each soil pit at depths 20, 45, 75, and 105 cm. Plate 3.1 shows a soil profile to 105 cm at the Basils site.

Plate 3.1 *Soil profile (ferrosol) at the Basils site. Note the abundance of roots at depth. The tape measure is in inches.*

3.2.4 Statistical analysis

Means were compared using an LSD where an ANOVA indicated a significant difference between means ($P \leq 0.05$). To determine the significance of changes in parameters over time within sites (e.g., for anaerobically mineralisable N and hot KCl extractable N) a 2-way ANOVA was used based on replicates and groups, where each individual mean to be compared was a separate group (eg. date-by-N extracted combinations). Comparing sites for average soil analysis values were by 1-way ANOVA using the SAS version 8 Proc Mixed program, data was $\sqrt{x}$ transformed to stabilise the variance. Correlations and regressions were determined by standard statistical methods.

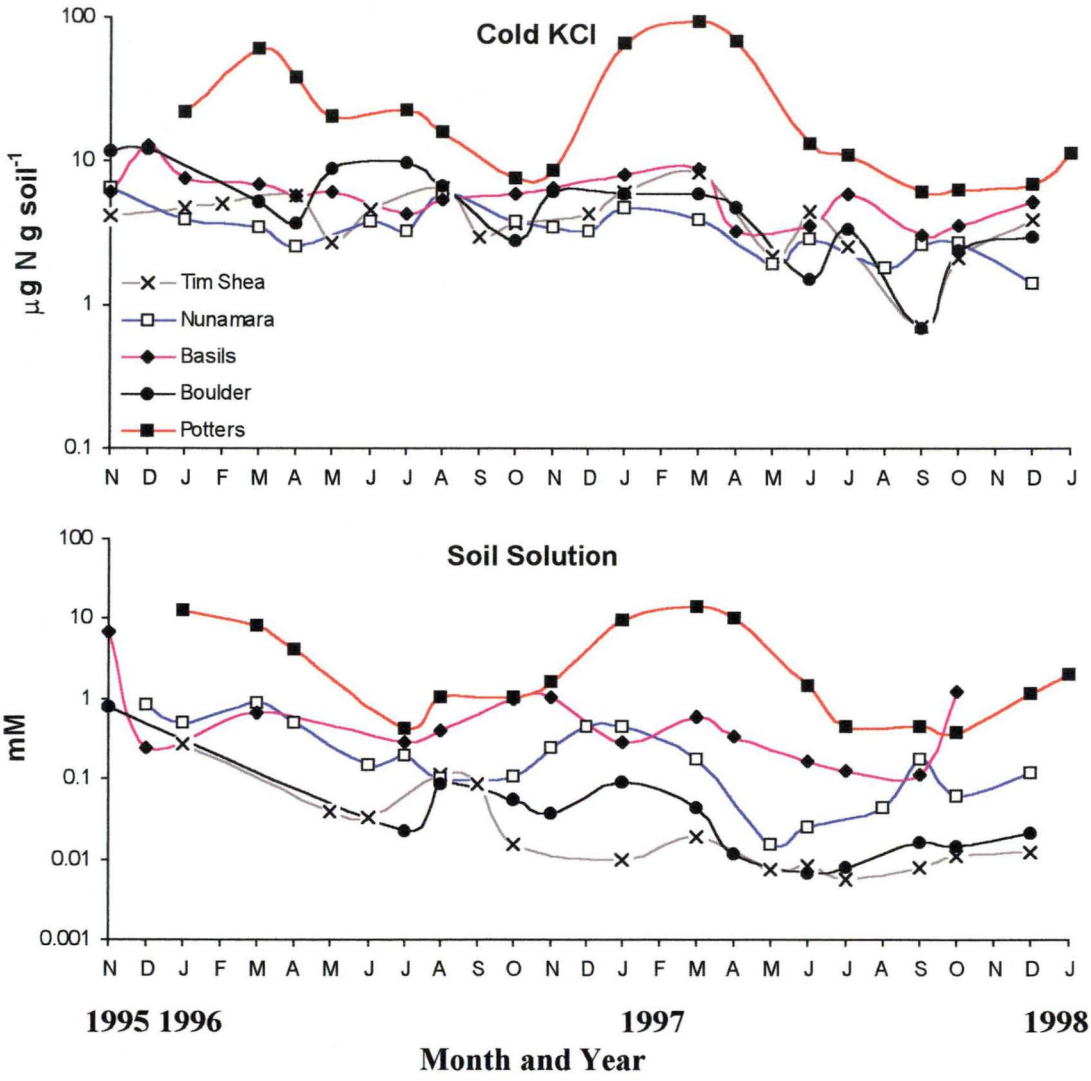
3.3 RESULTS

3.3.1 N mineralisation indices in surface soils

Soil solution and cold KCl extractable concentrations of NH_4^+ and NO_3^- ranged from <0.01-14.6 mM and <0.01-105.2 μg N per g soil, respectively, due to site and sampling date effects (Figure 3.1). These concentrations also had considerable short-term variation within sites. For example, at the Potters site between April 1997 and June 1997, soil solution NO_3^- dropped from 10.2 to 1.5 mM, and cold KCl extractable NO_3^- from 83 to 15 μg N g^{-1} soil. Average concentrations of cold KCl extractable and soil solution N and concentrations in January, April, July, or October were well correlated with annual NNM when the Potters site was included in the analysis. The Potters ex-pasture site, which had very high rates of NNM also had the highest concentrations of mineral N in cold KCl extracts and in soil solution except during spring each year (September-November). Values of r^2 for these correlations generally decreased when the Potters site was excluded (Tables 3.2 and 3.3). Soil solution N generally had higher correlations with annual NNM, with greater separation of sites, especially during the summer months (December to March) (Figure 3.1), than cold KCl extractable N. Extractable NO_3^- and extractable $NO_3^- + NH_4^+$ had higher r^2 values than extractable NH_4^+ when correlated with annual rates of NNM. Correlations (r^2) between log-log values of cold KCl extractable and soil solution NH_4^+ or NO_3^- were 0.30 or 0.43 for individual samples respectively (Figure 3.2). Ratios of soil solution

KCl extractable NO_3^- were close to 1 : 1, except for the Boulder site, but were lower for NH_4^+.

Figure 3.1. *Cold KCl extractable ($NH_4^+ + NO_3^-$) and soil solution ($NH_4^+ + NO_3^-$) from 1995 to 1998 at the Boulder, Nunamara, Tim Shea, Potters, and Basils sites.*



Anaerobically mineralisable N ranged from 30.9 µg N g^{-1} soil at the Nunamara site to 225 ug N g^{-1} soil at the Basils site, and significant differences between sampling dates occurred for all sites (Figure 3.3). Hot KCl extractable N ranged from 1.9 µg N g^{-1} soil at the Nunamara site to 199 µg N g^{-1} soil at the Basils site. Significant differences in sampling dates occurred at the Tim Shea site, but these were not consistent with

Table 3.2. *Coefficient's of determination (r^2) for linear relationships between average annual NNM rates and average values of soil analysis collected at the Boulder, Nunamara, Tim Shea, Basils and Potters sites.*

	+ Potters	-Potters	ex-forest sites
Total N	0.56	0.96	0.96
Total C	0.25	0.91*	0.93
Total P	0.85*	0.97*	1.00*
Hot KCl	0.58	0.90*	0.92
AMN	0.27	0.75	0.74
C:N	0.33	0.02	0.26
Cold KCl	0.85*	0.17	0.17
Soil solution	0.97**	0.94*	0.94

With the Potters site n=5, without the Potters site n=4, ex-forest sites n=3

AMN Anaerobically Mineralisable N

Ex-forest sites exclude the Potters and Boulder sites

** $P < 0.05$ ** $P < 0.01$*

seasonal patterns (Figure 3.4). Total N ranged from 0.22% at the Nunamara site to 0.74% at the Basils sites. Total P ranged from 0.05% at the Boulder site to 0.41% at the Potters site (Table 3.4). Total N, total P and hot KCl extractable N did not alter significantly with time within sites, except for hot KCl extractable N at the Tim Shea site. When comparing the average of values of anaerobically mineralisable N, hot KCl extractable N, total N and total P there were significant differences between sites in both years (Table 3.4).

Loss On Ignition (LOI) ranged from 9.4% at the Nunamara site to 31.3% at the Basils site. These data indicated total carbon to range from 3.6% at the Nunamara site to 13.7% at the Basils site. There were significant differences in total C with time at the Boulder, Nunamara, Tim Shea, and Potters sites, but these differences were not consistent with seasonal patterns. Only at the Boulder and Tim Shea sites were there a significant ($P < 0.05$) trends in total C with time, both sites demonstrating a slow decrease from the first estimates in October 1994 by Wang *et al.* (1996b) to

Table 3.3. *Coefficient's of determination (r^2) for linear relationships between average annual NNM and average soil analyses collected within 21 days of January, April, July and October of both years of measurement at the Boulder, Nunamara, Tim Shea, and Basils sites.*

N forms Extracted and Month	Soil Solution N		Cold KCl extractable N		Anaerobically Mineralisable N		Hot KCl Extractable N	
	A	B	A	B	A	B	A	B
January								
NH_4^+	0.67*	0.03	0.91**	0.22	0.28	0.78	0.57	0.92**
NO_3^-	0.91***	0.76	0.90**	0.06				
Total	0.91***	0.70	0.91**	0.17				
April								
NH_4^+	0.05	0.37	0.52	0.90**	0.45	0.63	0.63	0.86*
NO_3^-	0.98***	0.99***	0.92**	1.00#				
Total	0.98***	0.99***	0.90**	0.53				
July								
NH_4^+	0.06	0.07	0.27	0.23	0.32	0.81*	0.50	0.91**
NO_3^-	0.88**	0.87*	0.97***	0.97**				
Total	0.88**	0.86*	0.86**	0.04				
October								
NH_4^+	0.07	0.64	0.00	0.08	0.23	0.72	0.59	0.87*
NO_3^-	0.98#	0.87*	0.97***	0.94**				
Total	0.98#	0.87*	0.94***	0.80				

n = 3 for all sites except the Potters site where n = 5

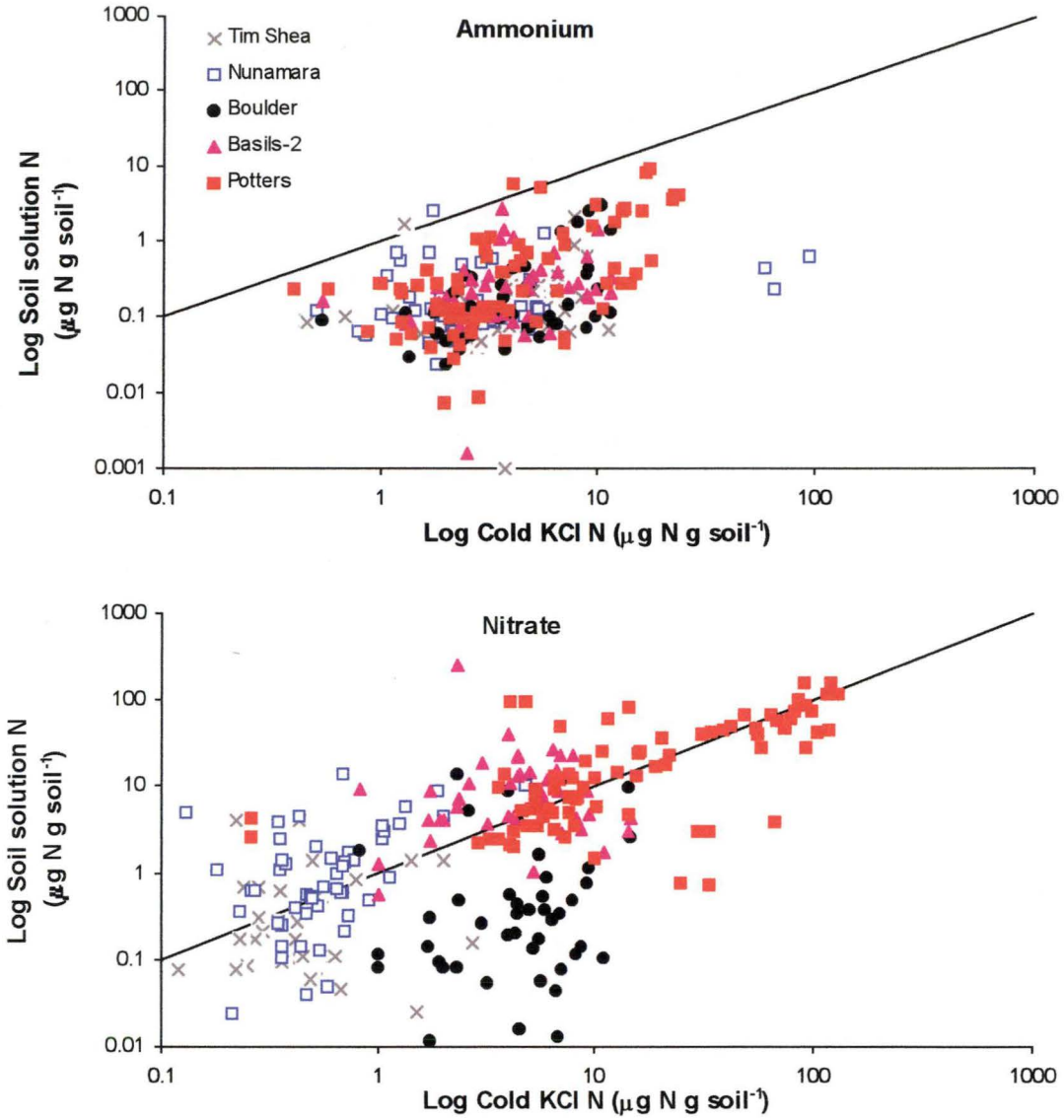
Total = ($NH_4^+ + NO_3^-$)

A including the Potters site B excluding the Potters site.

# P < 0.001, ***P < .01 ** P < 0.05, * P < 0.1

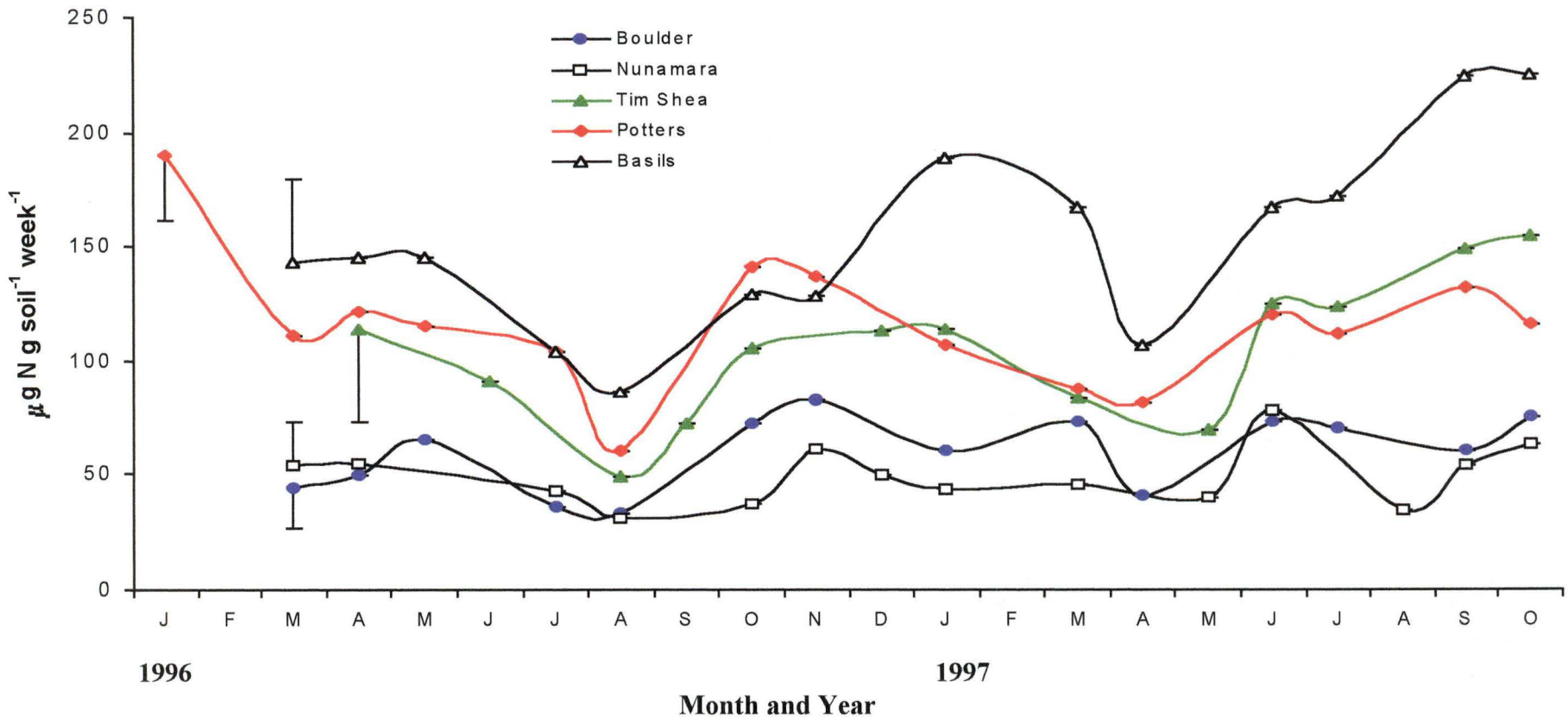
No result was obtained for January 1996 at the Boulder site, hence only 1997 data were used for January at this site.

Figure 3.2. *Relationship between soil solution NH_4^+ and NO_3^- and Cold KCl extractable NH_4^+ and NO_3^- at the Boulder, Nunamara, Tim Shea, Potters, and Basils sites on individual sample basis. Line represents 1 : 1 soil solution N : cold KCl extractable N.*



November 1997, the end of this study (Figure 3.5). Ratios of C:N correlated poorly with NNM with or without the Potters site in the analysis (Table 3.2 and 3.3). The Potters ex-pasture site had the lowest C:N ratio of 14 and the Boulder ex-pine site had the highest C:N ratio of 26 (Table 3.4).

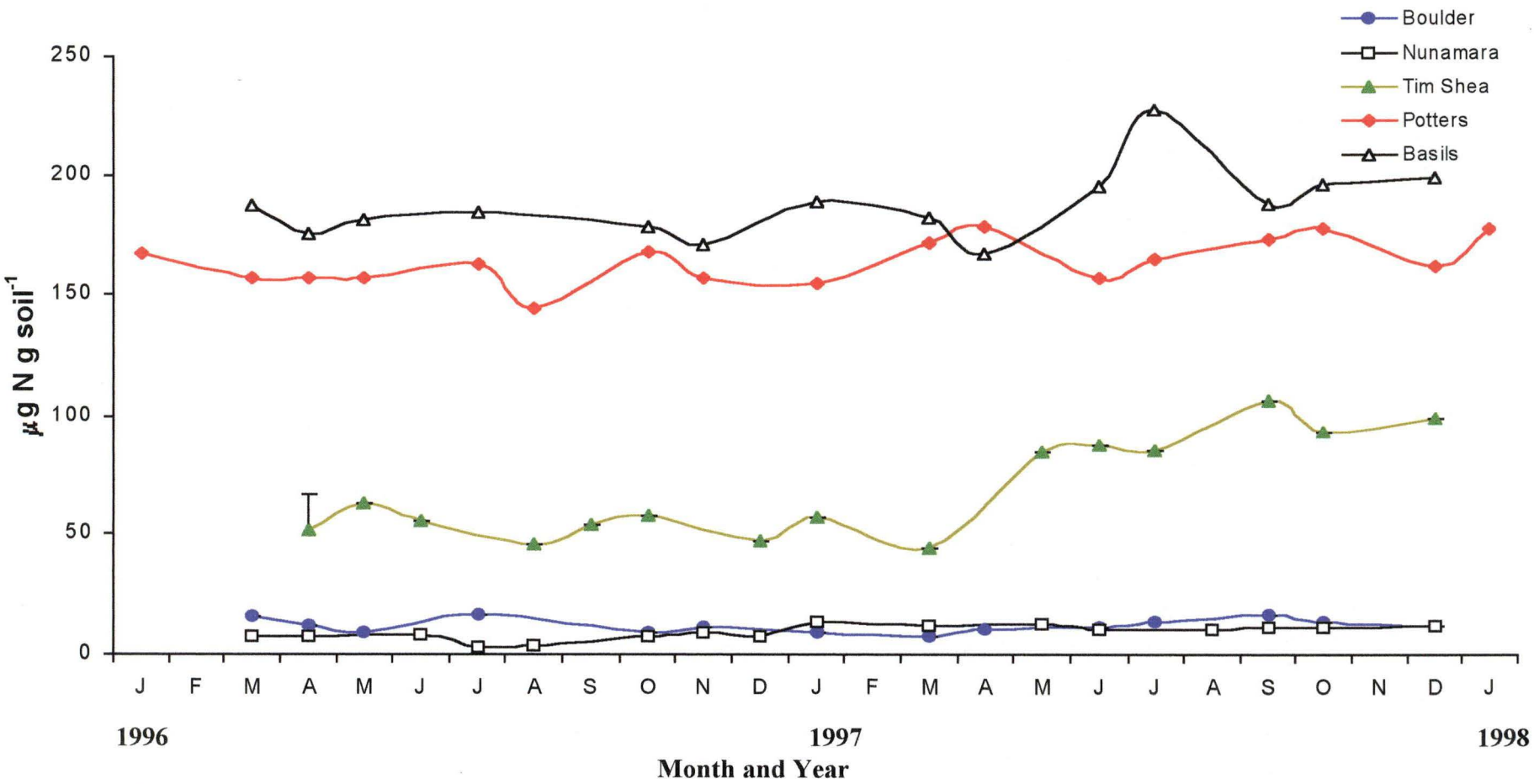
Figure 3.3. *Anaerobically mineralisable N (μg N g soil^{-1} week^{-1}) at the Boulder, Nunamara, Tim Shea, Potters, and Basils sites during the study period.*



Error bars are LSD where there were significant differences in sample period.

n=3 for all sites except the Potters site where n=5

Figure 3.4. *Hot KCl extractable N (μg N g soil^{-1}) at the Boulder, Nunamara, Tim Shea, Potters and Basils sites during the study period.*



Error bars are LSD where there were significant differences in sample period.

n = 3 for all sites except the Potters site where n = 5

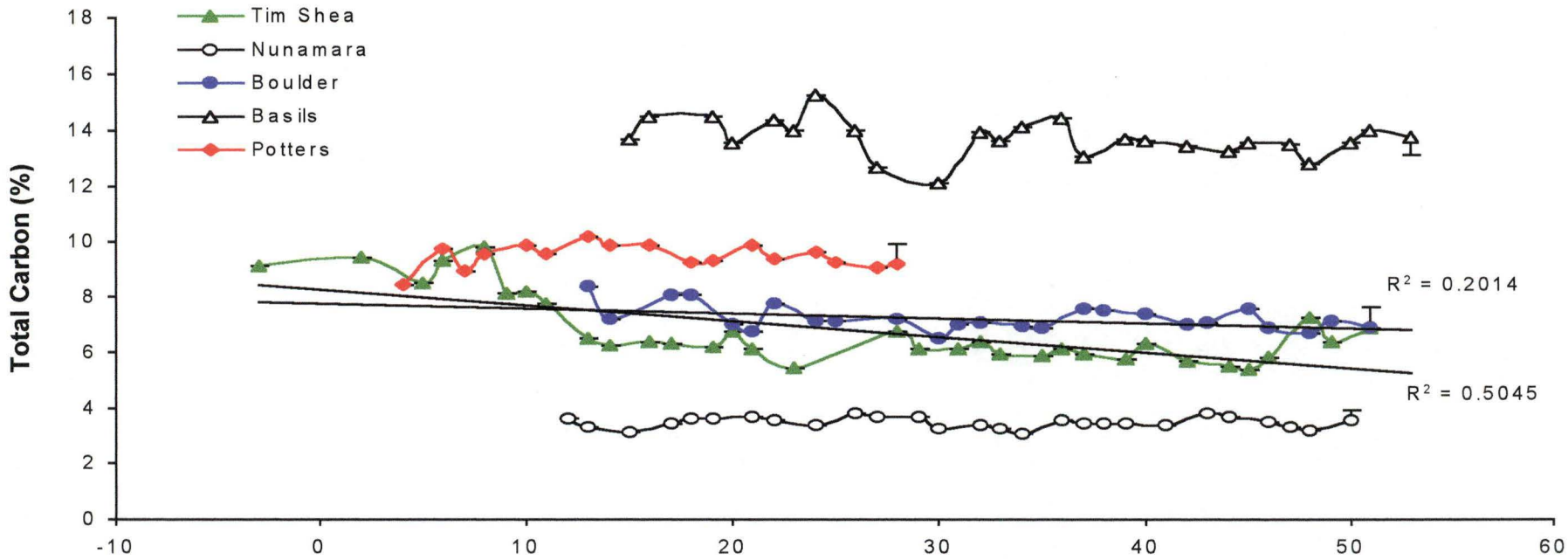


Figure 3.5. *Total soil carbon (%) with time at the five study sites. Regression lines and r^2 values are shown where significant ($p < 0.05$).*

Error bars are LSD, where there were significant differences with sample period ($P < 0.05$)
$n = 3$ for all sites except the Potters site where $n = 5$

Average values for anaerobically mineralisable N and hot KCl extractable N collected annually or in January, April, July or October did not correlate well with annual NNM. Total P, total C and total N also did not correlate well with annual NNM, however correlations (r^2) improved in all cases when the Potters site was removed from the regression (Tables 3.2 and 3.3). Correlations (r^2) of total C, total P and hot KCl extractable N with rates of NNM improved further when the ex-native forest sites were grouped. The correlation with total N remained constant and a drop of 0.01 was observed for the correlation with anaerobically mineralisable N (Table 3.2).

The Potters ex-pasture site had very high rates of NNM, but it was not distinguishable from the Basils site of lower rates of NNM by anaerobically mineralisable N, hot KCl extractable N, total N or total C. The Potters ex-pasture site was consistently ranked highest with total P, which ranked sites similar to NNM, ie Potters > Basils > Tim Shea ≈ Nunamara ≈ Boulder (Table 3.4).

Coefficients of determination for linear relationships between a range of soil analyses is presented in Table 3.5. Significant relationships exist between soil analyses total N, total C, hot KCl extractable N, total P and anaerobiaclly mineralisable N. These relationships are generally stronger when the Potters site is excluded from the regression.

The five study sites could be separated into two groups based on either hot KCl extractable N, total N, total P, or total C: those having NNM greater or less than 40 kg N ha^{-1} $year^{-1}$. Those of higher NNM had hot KCl extractable N > 100 μg N g^{-1} soil, total N > 0.4%, total P > 0.2%, and total C > 8% (Figure 3.6).

Table 3.4. *Average values for soil analyses for five* E. nitens *plantation soils, except where specified due to significant differences in sample periods. (P ≤0.05).*

	Site				
Soil Analysis	Boulder	Nunamara	Tim Shea	Potters	Basils
NNM[X]	24 c	23 b,c	13 c	188 a	70 b
NNM[Y]	13 c	20 c	23 c	175 a	76 a, b
A.M.N.[X]	57 cd	44 d	91 bc	119 ab	137 a
A.M.N.[Y]	66 c	51 c	110 b	108 b	172 a
A.M.N[Z]	62 c	43 c	101 b	113 b	154 a
Hot KCl	11[X] dc	10 c	52[X] b	164 a	185 a
Hot KCl	11[Y] b		65[Y] a		
Total N	0.27 b	0.22 b	0.33 b	0.65 a	0.74 a
Total P	0.05 b	0.08 b	0.08 b	0.41 a	0.32 a
Total C	7.0 c	3.6 d	6.1 c	9.4 b	13.7 a
C:N	26 a	16 bc	18 bc	14 c	19 b

n=3 at all sites except the Potters site where n=5

letters denote significant differences within years and soil analysis

NNM *values from chapter 2*

A.M.N. *Anaerobically Mineralisable Nitrogen.*

[X] *values for 1995/1996* [Y] *values for 1996/1997*

[Z] *average values for January 95/96 and 96/97*

Units: NNM (kg N ha^{-1} $year^{-1}$*)* *A.M.N. (μg N* g^{-1} *soil* $week^{-1}$*)*

Hot KCL (μg N g^{-1} *soil)* *total N, total P, total C (%)*

Table 3.5. *Coefficient's of determination (r^2) for linear relationships between average values of soil analysis collected at the Boulder, Nunamara, Tim Shea, Basils and Potters sites.*

a) *including the Potters site*

	1	2	3	4	5	6	7
1. Total N							
2. Total C	0.86**						
3. C:N	0.16	0.004					
4. A.M.N.	0.56	0.84**	0.10				
5. Hot KCl	0.98#	0.80**	0.24	0.88**			
6. Cold KCl	0.19	0.03	0.23	0.03	0.20		
7. Soil solution	0.39	0.13	0.35	0.14	0.42	0.94#	
8. Total P	0.87**	0.55	0.36	0.56	0.88**	0.49	0.73*

b) *excluding the Potters site*

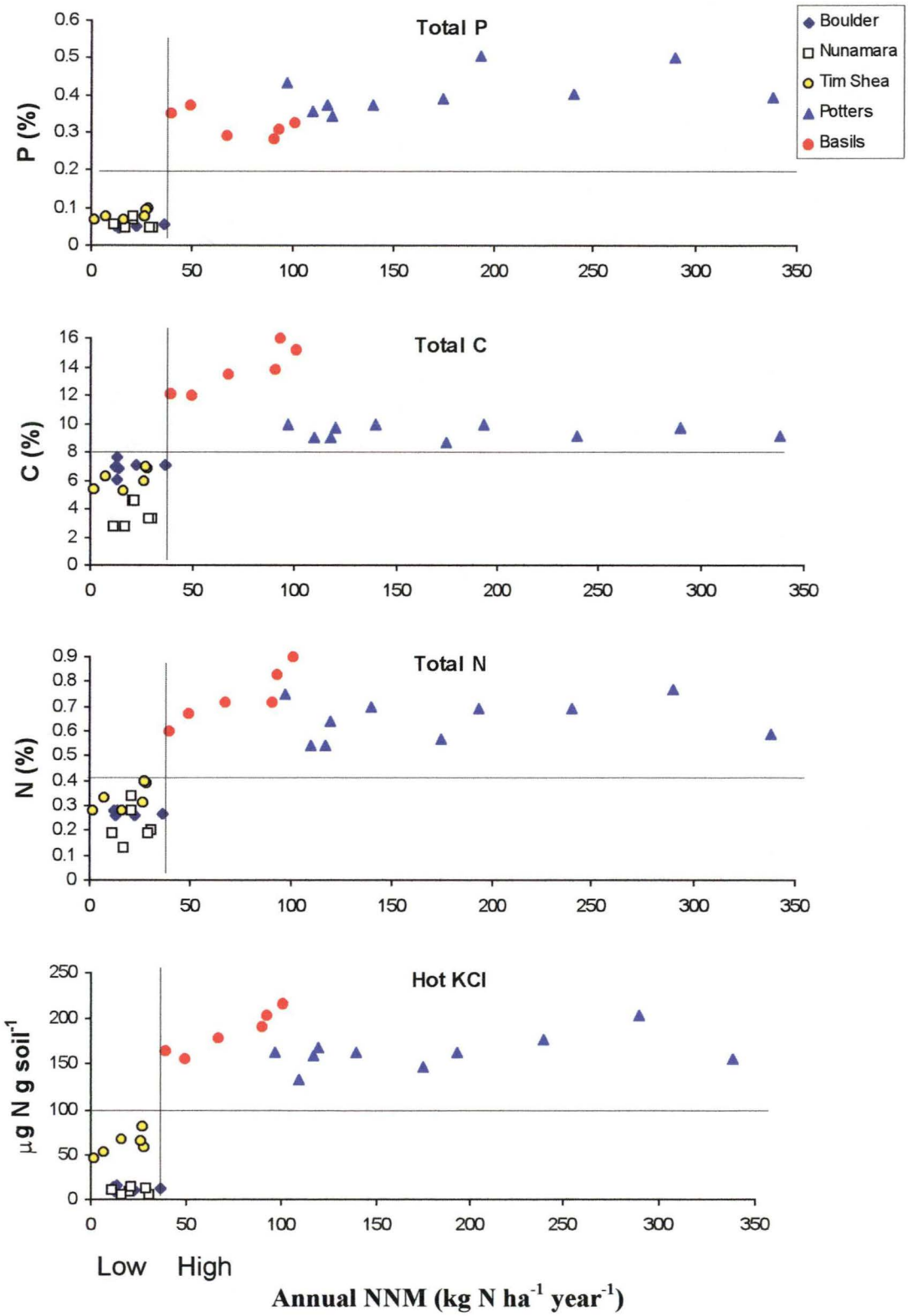
	1	2	3	4	5	6	7
1. Total N							
2. Total C	0.94**						
3. C:N	0.03	0.004					
4. A.M.N.	0.89*	0.83*	0.05				
5. Hot KCl	0.98**	0.88*	0.08	0.95**			
6. Cold KCl	0.27	0.11	0.65	0.46	0.40		
7. Soil solution	0.83*	0.73	0.06	0.54	0.76	0.12	
8. Total P	0.95**	0.83*	0.10	0.78	0.93**	0.30	0.93**

n=3 for all sites but the Potters site where n=5

A.M.N Anaerobically Mineralisable N

# $P < 0.001$, ** $P < 0.05$, * $P < 0.1$

Figure 3.6. *Separation of the Boulder, Nunamara, Tim Shea, Potters, and Basils sites into high and low annual NNM using total N, total C and total P, hot KCl extractable N values. n=3 for all sites except the Potters site where n=5.*



3.3.2 N mineralisation indices in subsoils

Concentrations of anaerobically mineralisable N, hot KCl extractable N, total N, and total C decreased exponentially with increasing soil depth at the Potters, Basils and Boulder sites with the exception of a slight increase in all soil analyses at the Boulder site from 45 to 75 cm depth. With depth, total P increased slightly at the Basils site, decreased slightly at the Boulder site and decreased exponentially (after an initial slight increase) at the Potters site (Figure 3.7). Compared to values for the 0-10 cm depth, contributions of anaerobically mineralisable N, Hot KCl extractable N, total N, total C and total P below 10 cm were large. Soil analyses values from 10 to 120 cm at the Potters and Basils sites and 0 to 90 cm the Boulder site were compared to those of the 0-10 cm depths, assuming samples at the 20, 45, 75, and 105 cm depths were representative of depth intervals 10 to 30, 30 to 60, 60 to 90, and 90 to 120 cm, respectively, and that bulk density of the soil profile did not decrease with depth. The contributions of subsoil to these analyses were, for the Potters, Basils and Boulder sites, respectively, at least 2.2 to 6.5, 2.1 to 9.8, 1.9 to 5.5 times that of the top 10 cm (Table 3.6). These estimates are likely to be slight underestimates due to the missing data from shallow soil pits and bulk density is likely to increase with depth. Total P and total C resulted in the largest estimates of subsoil NNM, however, soil analyses extracting N, total N, hot KCl extractable N and anaerobically mineralisable N indicated subsoil NNM was 1.9 to 2.9 times that of the top 10 cm.

At the Basils and Boulder sites cold KCl extractable and soil solution N decreased with depth (Figure 3.8), but at the Potters site there were large amounts of mineral N, found at 75-105 cm depths (289.3 $\mu g\ N\ g^{-1}$ soil cold KCl extractable; 2.8 mM N in soil solution). The proportions of mineral N extracted as NO_3^- in soil solution were 98 to 99% at the Potters site, 93% decreasing to 45% with increasing depth at the Basils site, and 35 % over 0-45 cm depths, decreasing to 17% at the 75 cm depth at the Boulder site.

Figure 3.7. *Anaerobically mineralisable N, hot KCl extractable N, total N, and total P at 0-10, 20, 45, 75 and 105 cm depths. Bars denote standard deviations.*

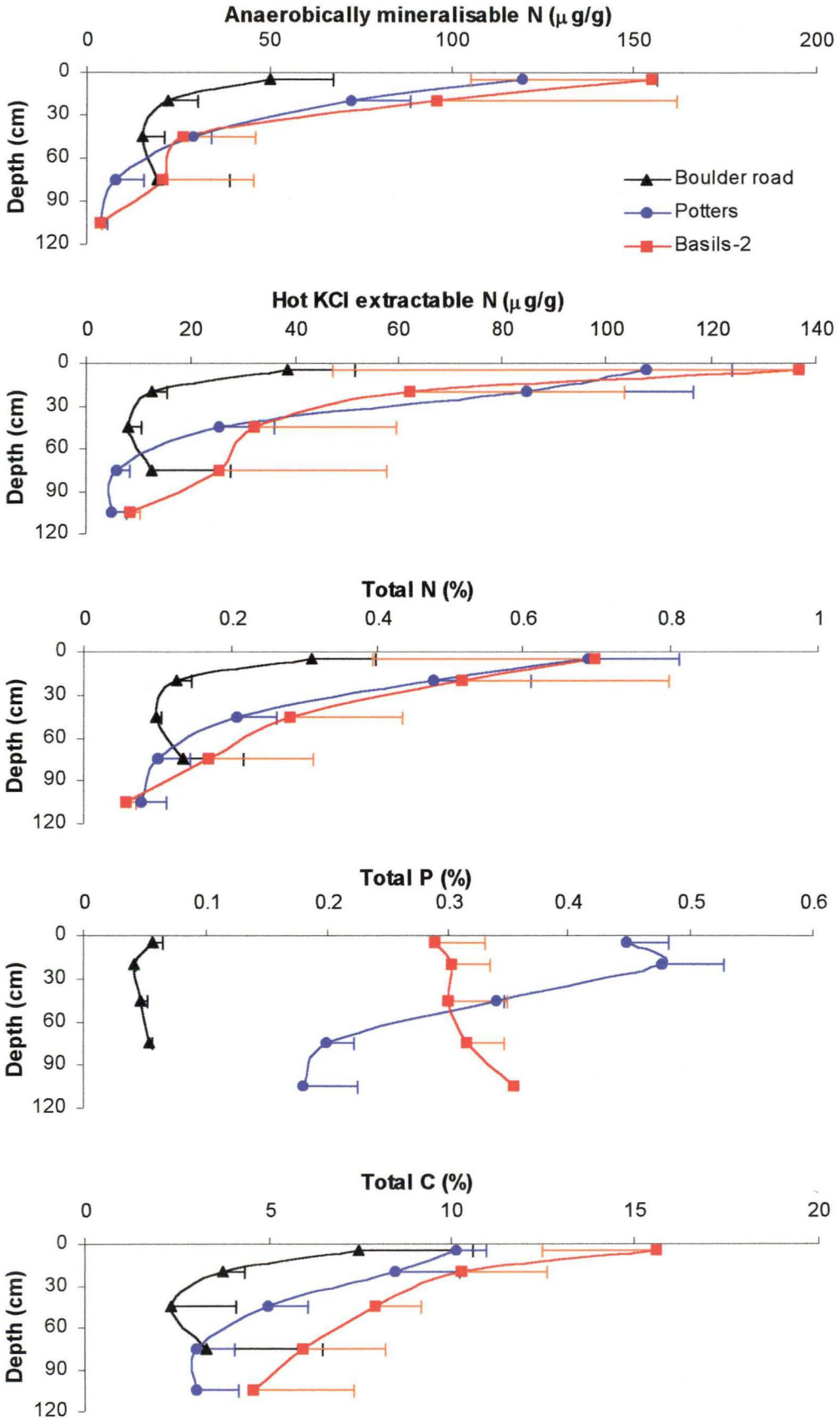
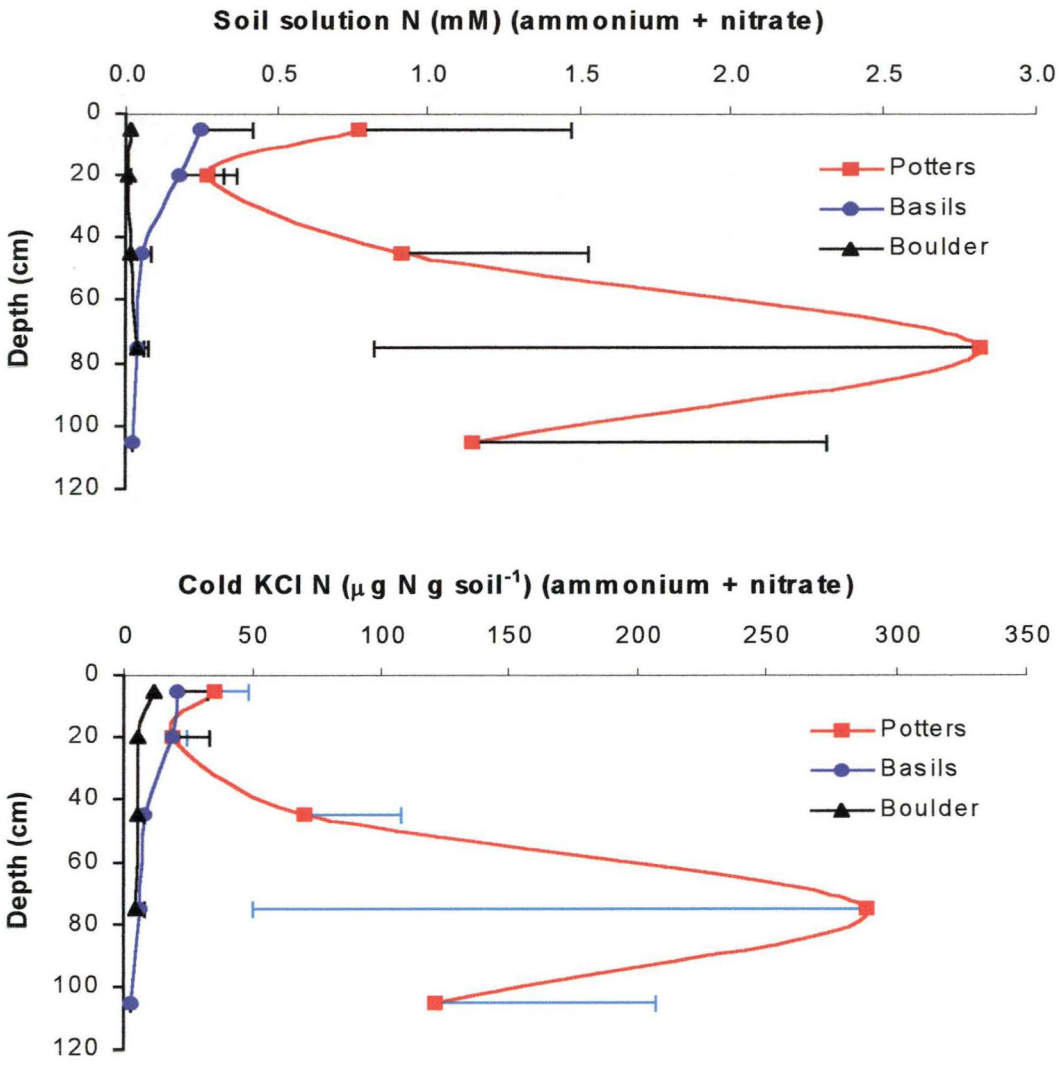


Table 3.6. *Soil analyses values from surface soils and subsoils at the Potters, Basils and Boulder sites. Standard deviations are shown in parenthesis. n=3*

Site and	Soil analyses				
Depth (cm)	Total N	Total P	AMN	Hot KCl	Total C
Potters					
[A]0-10	0.7 (0.1)	0.5 (0.0)	120 (37)	108 (16)	10 (1)
[B]10-120	2.0 (0.6)	2.9 (0.5)	262 (66)	273 (101)	47 (12)
B/A	2.9	6.5	2.2	2.5	4.6
Basils					
[A] 0-10	0.7 (0.3)	0.3 (0.0)	155 (50)	137 (90)	16 (3)
[B] 10-120	2.3 (1.1)	2.9 (1.3)	319 (246)	288 (233)	65 (22)
B/A	3.3	9.8	2.1	2.1	4.2
Boulder					
[A] 0-10	0.3 (0.1)	0.1 (0.01)	50 (18)	39 (13)	8 (3)
[B] 10-90	0.8 (0.3)	0.3 (0.1)	128 (63)	75 (39)	21 (3)
B /A	2.6	5.5	2.6	1.9	2.8

AMN = Anaerobically Mineralisable Nitrogen

Figure 3.8. *Soil solution ($NH_4^+ + NO_3^-$) and cold KCl extractable ($NH_4^+ + NO_3^-$) N at the Boulder, Basils, and Potters sites in September 1997 at 0-10, 20, 45, 75, and 105 cm depths. Bars denote standard deviations.*



3.4 DISCUSSION

Values of potential indices of N supply and *in situ* rates of NNM in this study covered a very wide range and were therefore a good basis for evaluating indicators of N supply.

Compared to the high rates of NNM at the Potters site, concentrations of anaerobically mineralisable N, hot KCl extractable N, total N, total P, and total C were disproportionately low (Table 3.4). Hence, the inclusion of the Potters site strongly affected relationships of NNM with total N and especially total C. All sites had similar temperature environments where long term average monthly temperatures did not vary more than 5 °C. All sites also received moderate to high rainfall (1000-1913 mm year^{-1}), and therefore did not experience significant periods of water stress (chapter 2). Hence, differences in *in situ* rates of NNM were unlikely to be due to environmental differences between sites, but largely due to differences in the quality and quantity of organic matter. Annual NNM rates corresponded to 3.4-3.6, 1.4-1.6, 0.7-1.0, 0.7-1.0 and 0.5-0.9% of total N (0-10 cm) at the Potters, Basils, Boulder, Nunamara, and Tim Shea sites, respectively, and are indicative of proportions of labile organic N. These data indicate that relationships between *in situ* rates of NNM and simpler chemical analyses will be specific to classes of previous vegetation and management. By inference then, the chemical analyses tested did not adequately indicate the differences in organic matter quality that resulted in the high rates of NNM on the ex-pasture site. Other authors also have suggested that differences in organic matter quality due to previous vegetation may not be accounted for by these soil analyses (Bremner 1965; Gonzales-Prieto *et al.* 1994; Juma and Paul 1984).

These data are the first to quantify the rate of NNM on an ex-pasture site in Tasmania. Although the Potters ex-pasture site had the highest proportions of total N mineralised, highest concentrations of total P, and the lowest C:N ratio, it also had the highest rates of NNM despite lower concentrations of total N and total C than the Basils site (ex-native forest). The contrasts between these two sites, therefore, are indicative of changes in soil following conversion of native forests to pasture that are well recognised (Skinner and Attiwill 1981), ie. phosphate fertilisers are added and N

supply is increased by a combination of decreased C:N ratios and an increase in concentrations of labile N due to N inputs through biological N fixation by legumes.

Although there were significant differences in ranking and poor linear correlations of NNM and soil analysis it was possible to separate the five sites described in chapter 2 into those having NNM greater or less than 40 kg N ha^{-1} $year^{-1}$ using hot KCl extractable N, total N, total P, and total C, where sites with NNM > 40 kg N ha^{-1} $year^{-1}$ had hot KCl extractable N greater than 100 μg N g^{-1} soil, total N greater than 0.4%, total P greater than 0.2%, and total C greater than 8%. For all soil analyses, the Potters site (ex-pasture site) ranked in the group of greater NNM and the Boulder site (ex-pine) ranked in the group of lower NNM, as suggested by Wang *et al.* (1996a). Wang *et. al* (1996a) found ex-forest sites ranked between ex-pasture and ex-pine sites for concentrations of total N, anaerobically mineralisable N, and hot KCl extractable N, but this was not always the case for the Basils, Tim Shea, and Nunamara ex-forest sites (Table 3.4).

Removing the ex-pasture site improved correlations of anaerobically mineralisable N, total P, total N, total C and hot KCl extractable N with rates of NNM. Further improvements in all except anaerobically mineralisable N occurred when the ex-native forest sites were grouped. Hence grouping sites by previous vegetation may improve correlations of these soil analyses with rates of NNM, in a similar way to grouping sites by primary profile form (Connell *et al.* 1995) or into strongly and weakly nitrifying soils (Carlyle *et al.* 1990) improved correlation's of total N with rates of NNM. Alternatively these soil analyses may be used as parameters in a model to predict rates of NNM similar to the way to O'Connell and Rance (1999) used anaerobically mineralisable N to predict rates of NNM in Western Australian forest soils.

Relationships between total N, hot KCl extractable N and total C with and without the Potters site, show strong relationships (r^2 = 0.80 - 0.98), suggesting that amounts of total N and hot KCl extractable N were dependent upon the quantity of organic matter present. Strong correaltions (r^2 = 0.78 - 0.95) existed between these soil analyses, total P and anaerobially mineralisable N only when the Potters site was excluded. Other

studies have also shown good correlations between these soil analyses, for example, between hot KCl extractable N and anaerobically mineralisable N (Gianello and Bremner 1986a, Aiman, 1992, Selmer-Olsen 1981), and between total N and anaerobically mineralisable N (Gianello and Bremner 1986b, Fox and Piedielek 1984). Stockdale and Rees (1994) found ^{15}N extracted anaerobically and with hot KCl were not well correlated. Hence, it is unlikely that hot KCl extractable N and anaerobically mineralisable N are from exactly the same pool of organic N.

The five sites could not be separated into groups based on anaerobically mineralisable N, irrespective of whether values were from samples collected in January, April, July, or October or were average values of each years sample periods. Anaerobically mineralisable N varied by more than a factor of two at all sites with sampling date, probably due to varying environmental conditions. This finding is in contrast to Polglase *et al.* (1992) who found little seasonal or annual variation in anaerobically mineralisable N but is in agreement with Adams and Attiwill (1986) and Powers (1980) who reported higher concentrations of anaerobically mineralisable N in the summer months. Anaerobically mineralised N is thought to come from microbial biomass N, which has been shown to fluctuate with season (Boone 1992) and soil temperature (Adams and Attiwill 1986; Powers 1980). Fluctuations in soil environment as well as variations in organic matter quality may have contributed to the poor separation of sites by annual NNM rates using anaerobically mineralisable N results.

Examination of cold KCl extractable N and soil solution N alone was not sufficient to group sites by NNM, these parameters varying considerably with sampling date. Ammonium can be nitrified, and NO_3^- is readily leached and denitrified. Both NH_4^+ and NO_3^- are subject to uptake by plants and immobilisation by soil microbiota. Hence, levels of mineral N are unlikely to accurately reflect NNM rates in all conditions, making them unreliable as predictors of NNM. Keeney and Bremner (1967) came to similar conclusions, and Rice and Havlin (1994) suggest that measurements of soil inorganic N are unlikely to provide predictions of NNM, but they may provide an estimate of soil N sufficiency at the time of sampling. However the Potters ex-pasture site had very high rates of NNM and the highest concentrations

of mineral N (except soil solution N in October 1997), hence soil solution and cold KCl extractable N may be useful for indicating sites of very high rates of NNM.

When soil solution N and cold KCl extractable N from individual sampling periods at the Boulder, Nunamara, Tim Shea, Potters, and Basils sites were plotted against each other, with results for both in $\mu g\ N\ g^{-1}$ soil, high correlations for extracted NO_3^- were expected with a slope close to one, as negligable interaction of NO_3^- with the soil is expected in surface soils (Black and Waring 1976). Correlations with NH_4^+ were not expected to be as high due to interactions of NH_4^+ with cation exchange surfaces. Displacement of NH_4^+ by K^+ from KCl, should result in more NH_4^+ being detected by KCl extracts. The ratio of NO_3^- extracted in KCl and soil solution was approximately 1:1 and more NH_4^+ was extracted using KCl extraction than in soil solution as expected. However, correlations of both NH_4^+ ($r^2 = 0.30$) and NO_3^- ($r^2 = 0.43$) extracted by cold KCl and soil solution extracts were lower and particularly poor at low concentrations (Figure 2). Cold KCl extraction was the first procedure conducted on the sieved soil; the commencement of the soil solution N assay was delayed for up to one week after cold KCl extraction. Potential effects of removal of the soil from the cool room (4 °C) to room temperatures and disturbance encountered during sampling for earlier procedures may have diminished reliability of soil solution N relative to cold KCl extractable N.

Net N Mineralisation and soil analyses are expected to decrease exponentially with depth (Persson and Wiren 1995; within the study soils Wang *et al.* 1988). Hot KCl extractable N, anaerobically mineralisable N, and total N values from 20-105 cm depths do decrease exponentially. However these subsoil soil analyses values indicate the N extracted from 10-120 cm depth represent up to three times the N extracted in the 0-10 cm depth. Of these soil analyses the microbially mediated anaerobically mineralisable N is the most likely to account for effects of depth on the mineralising biota. Using this soil analysis, the contribution of extractable N in the 20-120 cm depths was twice that of the top 10 cm.

Soil analyses from soil in the top 10 cm of the soil pits differed from those obtained during regular sampling of surface soils for the same depth. This is likely due to

reduced sampling of surface soils for the depth analysis. Sampling was reduced from eight sampling positions per plot during regular surface soil sampling to one sampling position per plot (from the top of the soil pit) for the depth analysis.

Concentrations of mineral N (comprising 98% NO_3^-) were very high at 75 cm depth at the Potters site (cold KCl extractable N was equivalent to 521 kg N ha^{-1} for 60-90 cm depth, assuming constant BD with depth and soil at 75 cm is representative of the 60-90 cm depth zone). This is probably the result of NO_3^- leaching, as indices suggest low rates of NNM at depth. High rates of leaching probably resulted from cessation of N uptake by the pasture in combination with high rates of NNM, nitrification and rainfall (chapter 2). Large amounts of mineral N have also been recorded at depth in agricultural ferrosols of the Tasmanian north west coast. For these soils 100-300 kg N ha^{-1} has been found at depths 40-100 cm, in some cases representing higher amounts of N than those found in surface soils (Sparrow pers. comm.). Within the ferrosols NO_3^- leached from the top 10 cm may be held on anion exchange at depth (Black and Waring 1976; Gillman and Abel 1987) reducing leaching losses. As the plantation's root system develops and N demand increases, less N is likely to be leached from surface soils, however, it is unknown to what extent mineral N accumulated at depth is later taken up by plantations.

In summary this study has confirmed several trends previously established by Wang *et al.* (1996a, 1998):

- The ex-pasture site (Potters) had high values of anaerobically mineralisable N, hot KCl extractable N, and total N. In contrast the ex-pine site (Boulder) had low values for all these parameters.
- Contributions of subsoil hot KCl extractable N, anaerobically mineralisable N, and total N were large compared to values in the top 10 cm.

There are also several important new findings for these soils.

- Hot KCl extractable N, total N, total C and total P were able to separate five sites into those having NNM greater or less than 40 kg N ha^{-1} $year^{-1}$.

- The ex-pasture site (Potters) had high values and the ex-pine site (Boulder) had low values for total C and total P.
- Anaerobically mineralisable N, soil solution N and Cold KCl extractable N varied considerably with time.
- Hot KCl extractable N, total N, total P, and total C were temporally stable during the November 1995-January 1998 study period.

4. ATMOSPHERIC INTERCHANGE OF NITROGEN

4.1 INTRODUCTION

Exchange of N with the atmosphere occurs through inputs in rain (from aerosols), which may be N enriched through the interaction with the above-ground biomass in throughfall and stemflow, N fixation, and losses from denitrification. These fluxes may need consideration when forming a N budget.

Inputs of aerosol nitrogen via rainfall can be as high as 20 kg ha^{-1} $year^{-1}$ in Europe and North America (Attiwill and Leeper 1987). However, for southern Australia, inputs range from 0.3 to 8.2 kg ha^{-1} $year^{-1}$ (Attiwill and Leeper 1987; Attiwill and Adams 1993; Baker 1982; Baker and Attiwill 1981, 1985; O'Connell 1985, Adams and Attiwill 1986; Flinn *et al.* 1979; Probert 1976; Weteslar and Hutton 1963; Bell and Barry 1980) and within Tasmania 3.7 to 8.2 kg ha^{-1} $year^{-1}$ (Adams and Attiwill 1991). For Tasmania, Adams and Attiwill (1991) found N input from rain tended to increase with distance from the coast, hence the study sites, which are all greater than 15 km from the coast, are likely to receive closer to the maximum N input of 8.2 kg ha^{-1} $year^{-1}$.

Throughfall and stemflow represent indirect atmospheric inputs of N to the soil via precipitation. Throughfall N is the N enrichment of rain as it passes through the canopy, stemflow is the N enrichment of rain as in runs down the stem. Bormann *et al.* (1977) estimated throughfall and stemflow in English northern hardwood forests to be 9.3 kg N ha^{-1} $year^{-1}$. *Eucalypt* forests of south-eastern Australia transferred 3.3-7.2 kg N ha^{-1} $year^{-1}$ in throughfall (Adams and Attiwill 1986) and *E. Obliqua* forest of SE Australia transferred 0.4-0.6 kg N ha^{-1} $year^{-1}$ in stemflow and throughfall (Baker 1982; Baker and Attiwill 1985); in north-east Tasmanian forests, throughfall ranged from 3.8-9.7 kg N ha^{-1} $year^{-1}$ (Adams and Attiwill 1991). Inputs of N to the soil from stemflow are usually less than a third and more commonly 10-20% of that in throughfall (Baker 1982; Parker 1983). Nitrogen fertilisation increases fluxes of N in throughfall (Parker 1983), hence throughfall at the study sites is likely to be toward the upper range found in *Eucalyptus* forests. At the study sites, throughfall and stem flow were not expected to exceed 10 kg N ha^{-1} $year^{-1}$ input to the soil from the standing biomass via precipitation.

Nitrogen fixation rates in improved pasture have been reported to be as high as 670 kg N ha^{-1} $year^{-1}$ (Sears *et al.* 1965), however, estimates have markedly reduced since ^{15}N methods were refined. For unimproved pasture, rates are more often less than 100 kg N ha^{-1} $year^{-1}$ (Whitehead 1995). For example, *Trifolium repens* fixed 11 to 18 kg N ha^{-1} $year^{-1}$ in grazed pastures of south-western Victoria (Riffkin *et al.* 1999). Symbiotic N fixation under forests is often much less, for example, legumes fixed 12 kg N ha^{-1} $year^{-1}$ under deciduous forests of south-eastern United States (Todd *et al.* 1978), and an average of 20 kg N ha^{-1} $year^{-1}$ under tropical forests of Latin America (Bentley *et al.* 1982). For Australia *Daviesia mimosoides* fixed 4.5 to 7.0 kg N ha^{-1} $year^{-1}$ under a mixed *Eucalypt* forest (McColl and Edmonds 1983); *Acacia dealbata* fixed 12-32 kg N ha^{-1} $year^{-1}$ under a two-year-old *E. regnans* plantation (Adams and Attiwill 1984); shrub legumes fixed 1.6 kg N ha^{-1} $year^{-1}$ under 6-year-old *E. marginata* regrowth forest (Hansen *et al.* 1987); and *Bossiaea laidawianna* fixed 1-14 kg N ha^{-1} $year^{-1}$ under *E. diversicolor* forest (Grove and Malajczuk 1992). There were no legumes observed at the present study sites, hence symbiotic N fixation is expected to be minimal. Rates of asymbiotic N fixation are markedly lower than rates of symbiotic N fixation. For example, 0.39-1.08 kg N ha^{-1} $year^{-1}$ has been reported for the litter layer from American Pacific NW forests (Heath *et al.* 1988); 0.3 kg N ha^{-1} $year^{-1}$ in forests from British Colombia (Cushon and Feller 1989); 0.3 to 3.8 kg N ha^{-1} $year^{-1}$ from Swedish coniferous forests (Granhall and Lindberg 1978 and Granhall *et al.* 1980); 0.64 to 1.08 kg N ha^{-1} $year^{-1}$ for Australian pine and *Eucalypt* forests (Baker and Attiwill 1984); and 0.38-2.57 kg N ha^{-1} $year^{-1}$ from Western Australian jarrah and karri forests (O'Connell and Grove 1987). Hence, rates of asymbiotic N fixation were likely to be less than 4 kg N ha^{-1} $year^{-1}$ at the present study sites.

Estimated contributions of <10 kg N ha^{-1} $year^{-1}$ for rain, throughfall and stemflow, and N fixation are low when compared to fluxes of annual NNM of up to 188 kg N ha^{-1} $year^{-1}$ (Chapter 2) and annual uptake into the above ground biomass of up to 68.9 kg N ha^{-1} $year^{-1}$ (Chapter 5). Hence, at the study sites, contributions of N in throughfall and stemflow, rainfall, and N fixation were assumed to be 10, 9, and 4 kg N ha^{-1} $year^{-1}$ respectively.

Denitrification results in the loss of nitrogen from forest ecosystems (Davidson 1990) due to production of gaseous nitrogen by microbial reduction of nitrogenous oxides (Tiedje 1982). Substrate is nitrate (NO_3^-), which is reduced to the principal products

nitrous oxide (N_2O) and di-nitrogen (N_2). Denitrification is a specific metabolic process present in a limited number of genera, where nitrogenous oxides serve as respiratory electron acceptors, allowing them to grow anaerobically. The pathway of denitrification is: $NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$, (Stouthamer 1992; Mosier and Klemedtsson 1994). A recently reported Anaerobic Ammonium Oxidation (ANAMMOX) pathway bypasses NO_3^- where NH_3^+ $NH_2OH \rightarrow N_2$, but has only been described in sewage treatment processes (de Graaf 1995).

There are large populations of denitrifying organisms in arable soils and the potential for denitrification is immense in most field soils (Tisdale *et al.* 1993). Denitrifying populations are ubiquitous, responsible for denitrification in flooded, desert, temperate, and tropical soils (Wollum and Davey 1975). Denitrification will only occur, however, in the presence of denitrifying population, sufficient concentration of NO_3^- (or other N oxides), adequate supply of organic C (Drury *et al.* 1991), or another electron donor, and environmental conditions such as anoxia and suitable pH and temperature (Davidson 1990). Denitrification requires temperatures greater than 5-10 °C (Stanford *et al.* 1975; George and Antoine 1982; Singh *et al.* 1989), and is strongly inhibited by O_2, because O_2 inhibits synthesis and activity of denitrification enzymes (Tiedje 1982). Water filled soil pores are required for denitrification to reach maximum rates (Ambus and Christensen 1993; Pell *et al.* 1996).

The only measurable property known that is specific for denitrifiers is the process itself, ie. the consumption of NO_3^- or NO_2^- or the production of N_2O or N_2. Measurements of NO_3^- and NO_2^- consumption are insensitive and non-specific due to other fates of nitrogenous oxides, such as microbial immobilization and plant assimilation of NO_3^-. The production of N_2 is complicated by large concentrations of N_2 in the atmosphere. Hence, the production of N_2O provides the best indicator, being more commonly measured by using acetylene to prevent its conversion to N_2 (Tiedje 1982). Measurement of N_2O production is carried out by either covering soil *in situ* with an airtight container (e.g. Hilton *et al.* 1994) or by transferring soil to the laboratory (e.g. Dutch and Ineson 1990). In both cases, N_2O accumulation is measured in the head space of an incubation chamber using a gas chromatograph (Smith and Tiedje 1979) or by mass spectroscopy (Arah *et al.* 1993).

A common way to characterise denitrification in soil has been to determine potential denitrifying activity. This is done by providing soil samples with NO_3^-, a carbon source, anaerobic conditions, and a 20 °C temperature, making the concentration of denitrifying enzymes the rate-limiting factor. Pell *et al.* (1996) used a solution containing 1 mM KNO_3 and 1 mM glucose (electron donor), which is typical of the concentrations of nitrogen and glucose used by others. Anaerobic conditions can be created by flushing the jars with N_2 and problems associated with substrate diffusion can be overcome by use of a soil slurry (Pell *et al.* 1996; Myrold and Tiedje 1985; Ambus and Christensen 1993).

Denitrification rates from temperate forest soils tabulated by Davidson *et al.* (1990) fall in a range of <0.01 to 50 kg ha^{-1} $year^{-1}$ and Dutch and Ineson (1990) estimated up to 40 kg ha^{-1} $year^{-1}$ N was denitrified during the first two years after clear-felling an English spruce forest.

Data described in chapter 2, show NNM (0-10 cm depths) rates at the Basils site ranked 18 kg N ha^{-1} $year^{-1}$ and 15 kg N ha^{-1} $year^{-1}$ more than leaching for the November 1995-November 1996 and November 1996-November 1997 years respectively, without an increase in the mineral N content of soil collected in October 1996 to October 1997. There were high rates of nitrification at the Boulder, Nunamara, Tim Shea, Potters, and Basils sites resulting in high proportions of mineral N as nitrate, especially at the Potters and Basils sites (chapter 2), where soil water content was likely to be approximately field capacity from March to November for both years of measurement (chapter 2). Hence denitrification could potentially result in a significant N-loss at the study sites.

The experimental objective of work reported in this chapter was to estimate the significance of denitrification, for the budget approach, at two study sites known to have high rates of nitrification.

4.2 MATERIALS AND METHODS

4.2.1 Denitrification, unamended soil

Four soil cores (3 cm dia. and 10 cm deep) from the Basils site and three soil cores from the Potters site from the inter-row of each of three control plots were sampled in April 1998 and returned to the laboratory. The soil was removed from the cores with as little disturbance as possible and placed into individual 750 mL 'Le Parfeit' glass jars with 5 mL acetylene and sealed. Samples (0.1 mL) of head space gas were taken periodically for analysis as described below. The concentrations of NH_4^+ and NO_3^- in soil from each soil core were estimated by cold KCl extraction (as described in 2.2.4) immediately after the incubation period.

4.2.2 Potential denitrification, amended soil

Eight cores (5 cm dia. and 10 cm deep) from the inter-row of each of three plots at the Basils site were sampled in March 1997 and returned to the laboratory. Soil collected from each plot was mixed and 100 g (moist) sub-samples (59 g oven dry) were taken and placed into individual 750 mL 'Le Parfeit' glass jars and mixed with the following treatments:

1. 40 mL 1 mM NO_3^-
2. 40 mL 1 mM glucose
3. 40 mL 1 mM NO_3^- and 1 mM glucose
4. 40 mL water

Each treatment was repeated in triplicate. The jars were purged with N_2, injected with 30 mL acetylene, and sealed. Head space gas (0.1 mL) was sampled periodically for analysis as described below.

4.2.3 Analysis

Samples were analysed with a Hewlett Packard 5890 gas chromatograph connected to a Hewlett Packard 5970B mass spectrometer. Samples were injected in split mode and passed through 25 m, 32 mm internal diameter, Chrompak methyl silicone liquid phase (5 μm thick) column with a head pressure of 10 pounds per square inch. Column temperature was 25 °C with an injection temperature of 50 °C and split flow

of 85 mL per minute with 0.1 mL sample injected. A standard curve was constructed using changes in $^{29}N_2$: $^{30}N_2O$ ratios. The $^{29}N_2$ isotope of N_2 occurs in abundances low enough for comparison with other gasses at the ppm scale. To produce the standard curve, known concentrations of N_2O in N_2 (all isotopes) were injected into the mass spectrometer. The peak areas of the $^{29}N_2$ isotope of N_2 and the $^{30}N_2O$ isotope of N_2O formed the ratios used in the standard curve from which concentrations of N_2O evolved from the soil were measured.

For estimates of denitrification rates, soil cores of surface area 0.0010752 m^2 were used. Hence, estimates of denitrification rates per ha can be obtained by multiplying rates within the jars by 9300595 (10000/0.0010752). For estimates of potential denitrification the weight of oven dry soil (0-10 cm depth) used was 59.125 g. With soil bulk density at the Basils site of 0.65 (Table 2.1), one hectare to 10 cm depth contains 65 0000 kg of soil, hence estimates of denitrification rates per ha can be obtained by multiplying rates within jars by 10993658 (650000/0.059125). Concentrations (ppm) of the N_2O within the jars is $^{29}N_2$: $^{30}N_2O \times 145.87$ (from the standard curve, Appendix). Potential rates of denitrification N loss (kg N ha^{-1} $year^{-1}$) at the detection limit of 5 ppm N_2O at 6.75 h (1/129.7778 years) with the head space of the jars of 0.66L (0.75L-0.09L added soil and soil water) can be estimated as follows;

$0.66/1000000 \times 5 = 3.3E^{-6}$ L N_2O

$\therefore 3.3E^{-6} / 22.7 = 1.47\ E^{-7}$ moles N_2O

$\therefore 1.47\ E^{-7} \times 14 / 1000 = 4.13\ E^{-9}$ kg N

$\therefore 4.13\ E^{-9} \times 9300595.238 = 0.03865$ kg N ha^{-1}

$\therefore 0.03865 \times 129.778 = 4.98$ kg N ha^{-1} $year^{-1}$

4.2.4 Statistical analysis

Means were compared using LSD where an ANOVA showed a significant difference between means ($P \leq 0.05$). Correlations and regressions were determined by standard statistical methods.

4.3 RESULTS

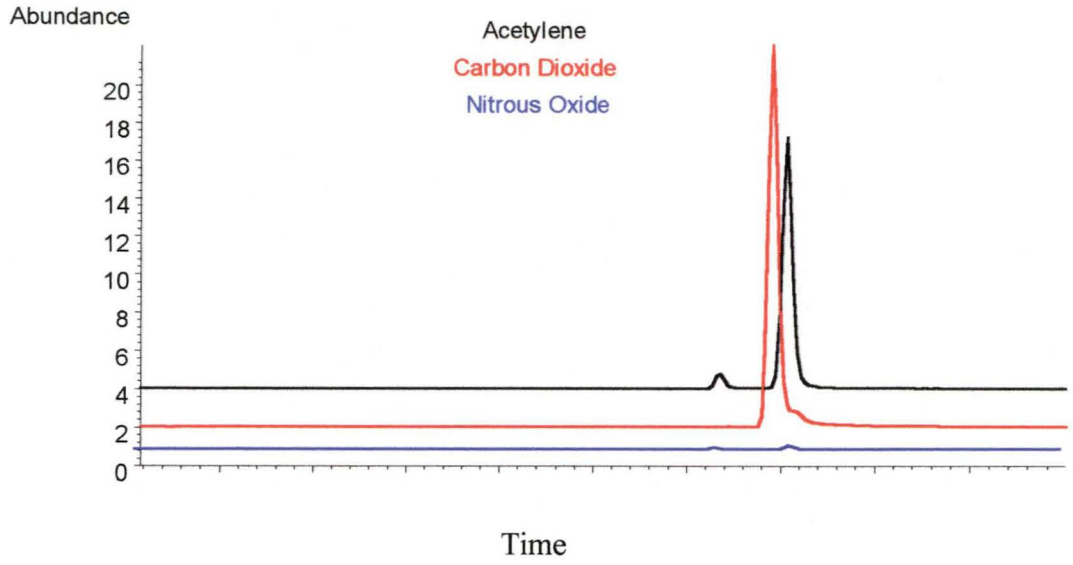
4.3.1 Denitrification, unamended soil

Evolution of N_2O from unamended soil after one day was below detection for the apparatus (< 5 ppm), hence, rates of denitrification were less than 5 kg N ha^{-1} $year^{-1}$ in unamended soils. Nitrate was present in incubated soil at cold KCl extractable concentrations of 14.4 μg N g^{-1} soil and 1.1 μg N g^{-1} soil at the Potters and Basils sites respectively, equivalent to 0.5 kg N ha^{-1} at the Basils site and 7.4 kg N ha^{-1} at the Potters site. Soil water content was 77% in the Basils soil and 56% in the Potters soil, which was above field capacity (-25 kPa), for both sites (Figure 2.1). Analysis for the acetylene inhibitor showed that acetylene was present throughout the incubation. An example trace for acetylene, nitrous oxide, and carbon dioxide are shown in Figure 4.1a and 4.1b, in which CO_2 concentrations in the head-space increased indicating microbial activity. Increasing CO_2 concentrations were indicated by increases in the $^{44}CO_2$: Ar ratio (Figure 4.2), Ar concentrations remained constant during the incubation. A N_2O peak was observed following injection of N_2O gas into the head space of jars at the end of the incubation.

4.3.2 Potential denitrification, amended soil

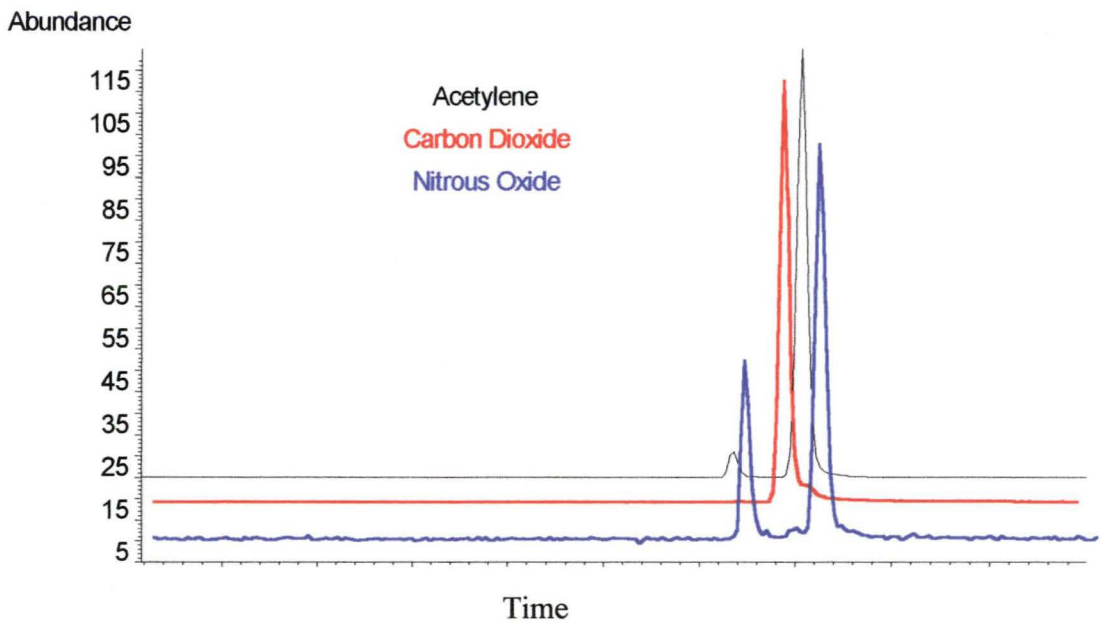
Rates of N_2O production after 6.75 hours were 4.7-9.2 × 10^{-5}g N per 59.125 g soil, which, when projected, is equivalent to annual rates of 676-1308 kg N ha^{-1} (Table 4.1). Note, potential denitrification is projected to annual rates for comparative purposes only and is not intended to signify actual field rates. Unamended soil had nitrate concentrations of 5.5 μg N g^{-1} dry soil and soil solution nitrate concentrations of 1.06 mM. The added 40 mL of 1 mM nitrate contained 6.5 μg N g^{-1} incubated soil.

Figure 4.1a. *Example of a gas chromatograph-mass spectrometer trace for relative abundance of acetylene, carbon dioxide, and nitrous oxide.*



Time and abundance are in arbitrary units

Figure 4.1b. *The above trace with normalised peak areas for acetylene, carbon dioxide, and nitrous oxide showing each trace set to an individual abundance scale.*



Time and abundance are in arbitrary units

Nitrous oxide has been off-set right to avoid overlap of peaks.

Figure 4.2. *$^{44}CO_2$: Ar ratio with time during potential denitrification incubation. Soils 1-12 are from the Potters site, soils 13-18 are from the Basils site.*

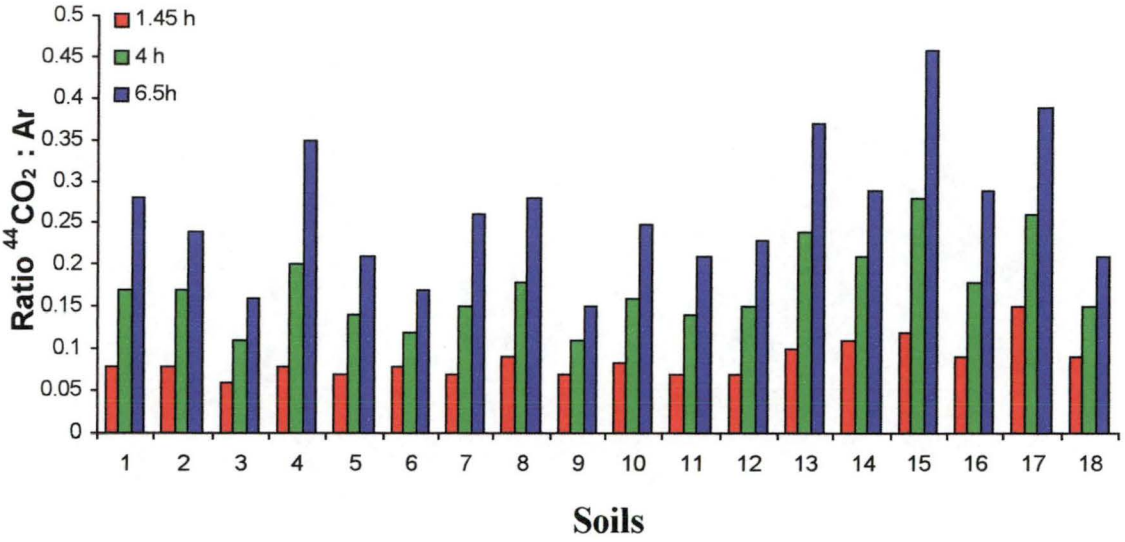


Table 4.1 *Potential denitrification rates at the Basils site for soil collected in March 1997*

Treatment.	N (10^{-5}g) per jar at 6.75 hours	Projected annual rate. (kg N ha^{-1})
1. 40 mL 1 mM NO_3^-	9.2	1308
2. 40 mL 1 mM glucose	4.7	676
3. 40 mL 1 mM NO_3^- and glucose	8.5	1212
4. 40 mL water	5.0	710

Significant differences ($P < 0.05$) 1 and 3 > 2 and 4.

4.4 DISCUSSION

From results reported in chapter 2, the Potters and Basils sites were seen to have the highest rates of annual NNM (175 to 188 kg N ha^{-1} and 70 to 76 kg N ha^{-1} respectively), nitrification (52 to 61 kg N ha^{-1} and 165 to 199 kg N ha^{-1} respectively) and soil mineral N contents (up to 93 kg N ha^{-1} at the Potters site). Hence they would also have the greatest potential for denitrification. Although soil disturbance and incubation 20 °C are conducive to enhanced denitrification, unamended soil collected from the Potters and Basils sites in April 1998 did not produce detectable amounts of N_2O during laboratory incubation. This corresponded to less than 5 kg N ha^{-1} $year^{-1}$ being denitrified (extrapolation of minimum detectable rate at 6.75 hours). Denitrification will be limited in winter by low temperatures (Table 2.1) and in summer by aeration and lower water contents (Figure 2.1). Hence denitrification rates are likely to be highest in autumn (March-April) when warmer temperatures occur in conjunction with high soil water content. However, at this time the Potters soil contained more nitrate than was added for estimation of denitrification potential and both the Basils and Potters sites had high soil water contents, yet neither produced detectable N_2O. Hence, rates of denitrification are likely to be low in the study sites.

Large potential denitrification rates were indicated for the Basils site (Table 4.1). Soil incubated anaerobically as a slurry in the absence of added NO^{3-} produced N_2O at rates equivalent to 710 kg N ha^{-1} $year^{-1}$ for the 0-10 cm depth. The addition of 1 mM glucose did not significantly alter rates, but the addition of 1 mM nitrate significantly increased denitrification potential by 84%. Under anaerobic/waterlogged conditions the potential for denitrification is large but well within potential rates found by other authors. For example 13 140 kg N ha^{-1} $year^{-1}$ by Binstock (1984) in forest soil and 35 000 kg N ha^{-1} $year^{-1}$ by Pell *et al.* (1996) in agricultural soils. Potential rates of denitrification were not restricted by carbon source but rather by nitrate, similar to Weir *et al.* (1993) but in contrast to Blew and Parkinson (1993), even though at the Basils site nitrate was present at 5.5 μg N g $soil^{-1}$. Though the surface soils (0-10 cm) at the Potters and Basils sites had undetectable rates of denitrification, these results indicate that under waterlogged conditions the potential for denitrification at the study sites is high. Waterlogged conditions were not observed at either site during the study period, but may occur after heavy rain or at depth. Due to variable temperature and moisture regimes encountered in the field (chapter 2) and their strong effects on rates

of denitrification, conclusive measurements of denitrification rates require an in situ method, such as Hilton *et al.* (1994) that were beyond the scope of this study.

In summary this study has shown the following important new findings:

- Denitrification rates were not detectable for soil collected in April 1998 at the Potters and Basils sites.
- Under waterlogged conditions, (which were not observed at either site during the study period), denitrification potential was high at the Basils site, and this potential was not restricted by carbon, but was restricted by nitrate.

5. UPTAKE OF NITROGEN BY FAST-GROWING *E. NITENS* PLANTATIONS IN TASMANIA

5.1 INTRODUCTION

Within Tasmanian *E. nitens* plantations, fast growing *E. nitens*, having the greatest increment in biomass, are expected to have the greatest demands for N. Acquired nitrogen is either stored in above- and below-ground biomass, or lost from the biomass through processes such as litterfall, stemflow, and throughfall. Estimates of N demand of different aged plantations may improve our understanding of plantation N requirements.

Measurement of tree biomass is labour- and time-intensive, hence biomass is often estimated from predetermined regression equations based on tree diameter and height. Estimates of N concentrations in plant components can be multiplied by their respective biomasses to estimate plantation N content. For example, for uptake of N into the above-ground biomass George (1985) estimated that an unfertilised 5-year-old *eucalyptus* hybrid plantation in India contained 229 kg N ha^{-1}; Bennett *et al.* (1997) estimated that a fertilised 6-year-old *E. globulus* plantation in Gippsland, Victoria, contained 230 kg N ha^{-1}; Cromer and Williams (1982) found that N accumulated in a fertilised *E. globulus* plantation in Victoria was 53.1, 92.1, 125.3, and 153.2 kg N ha^{-1} for ages 2, 4, 6, and 9.5 years, respectively; Birk and Turner (1992) estimated that a fertilised 9-year-old *E. grandis* plantation in New South Wales contained 246 kg N ha^{-1} and Misra *et al.* (1998b) found that N accumulated in two fertilised Tasmanian *E. nitens* plantations were 10, 130 and 290 kg N ha^{-1} for ages 10, 26 and 34 months respectively. These estimates of standing N content represent uptake rates into the above-ground biomass of 8-102 kg N ha^{-1} $year^{-1}$.

Misra *et al.* (1998b) report the below-ground N content of Tasmanian *E. nitens* plantations of ages 10-34 months to be 0.39 of above-ground N. This ratio is similar to that reported for a 5-year-old *Eucalyptus* hybrid grown in India where below-ground N was 0.31 of above-ground N (George 1985). Assuming below-ground N content of 0.39 applies to the above above-ground N uptake rates, N uptake rates into above- and below-ground biomass range from 11-142 kg N ha^{-1} $year^{-1}$. These rates of

uptake represent large proportions of annual NNM measured at the less N fertile study sites (the range of NNM rates encountered was 13-188 kg N ha^{-1} $year^{-1}$ [0-10 cm], chapter 2 with potentially 39-364 kg N ha^{-1} $year^{-1}$ [0-120 cm] chapter 3). If these N uptake rates are required to maximise growth at the study sites, those in the lower end of the NNM range (Tim Shea, Boulder and Nunamara) are likely to require N applications to achieve and maintain them.

Unpublished regression equations derived for 1- to 5-year-old *E. nitens* of diameters 0.2-15.4 cm at breast height (1.3 m over bark) and heights 142-1304 cm grown in SE Tasmania, were available to estimate the biomass of leaf, branch, and stem tissues (R Cromer pers. comm.). However, some trees within the study sites required an extended means of biomass estimation because they were larger than those covered by the Cromer equations. *Eucalyptus globulus* and *E. nitens* belong to the same sub-genus, *Symphomyrtus*. Hence, *E. globulus* is likely to have similar growth patterns to *E. nitens.* The Hingston and Galbraith (1998) biomass regression equations for SW Australian *E. globulus* plantation trees aged two to ten years, of diameters 3 to 30 cm at breast height and 3.6 to 29.4 m in height, encompassed the larger trees within the study sites. Hence, these equations were used to estimate biomass of stem wood, stem bark, branch, and leaf tissues of the larger trees at the study sites.

Tissue N concentrations in *Eucalyptus spp.* tend to be highest in leaves with intermediate values for bark and branches and lowest values for wood (Judd *et al.* 1996). Nitrogen concentrations in all tissues tend to be highest in young plantations and drop with age (Pereira *et al.* 1984; Beadle and White 1968; Attiwill 1980; Cromer and Williams 1982). For example, unpublished data for *E. nitens* plantations in Tasmania show decreases in average N-concentrations of 1- to 4-year-old plantations of 2.18% to 1.28% in leaves, from 1.03% to 0.41% in branches, from 1.07% to 0.56% in bark, and from 0.56% to 0.24% in stem tissues (R Cromer pers. comm).

Sampling of all plant tissues is time-consuming and not suitable for routine use in plantations. Nitrogen concentrations in leaves tend to decrease as plantations age and can vary with season (Pereria *et al.* 1994). It is possible that a point in the canopy exists which has the same N concentration as, or is highly correlated with, that in the

whole tree or individual components. Data presented by Leunig *et al.* (1991) suggest that for 6- and 16-month-old *E. grandis* such a point exists at 70% of canopy height where leaves have N concentrations representative of the whole canopy. A representative sampling position would greatly reduce effort required to estimate the average concentration of N in leaves, and other components.

For young plantations that have not closed canopy, and therefore, do not drop large amounts of litter, increases in the N content of above-ground and below-ground components provide an estimate of minimum N demand. However, as plantations age and their canopies begin to close, shaded leaves die and litter begins to accumulate. The uptake demand for N, lost from the standing biomass, in litter may be important when estimating total uptake demand of N in *E. nitens* plantations. The amount of N in litterfall for Australian forests covers a wide range. For example, stands of *E. regnans* in Victoria of ages 5-250 years returned 3.8-8.4 kg N ha^{-1} $year^{-1}$ in litter (Polglase and Attiwill 1992); *E. pauciflora, E. diversicolour* and *E. delegatensis* in Western Australian forests returned 16.7, 18.8, and 29.3 kg N ha^{-1} $year^{-1}$ in litter respectively (Woods *et al.* 1980); *E. diversicolor* regrowth forests of Western Australia returned 25.3-51.2 kg N ha^{-1} $year^{-1}$ in litter, where N fertilisation increased the amount of N falling in litter and the proportions of leaves in litter (O'Connell and Grove 1993). Attiwill and Leeper 1987 report a range of 9.8-130 kg N ha^{-1} $year^{-1}$ falling in litter for *Eucalypt* forests from various states. Litterfall in plantations is likely to be higher than native forests due to N fertilisation.

Of concern however, is the possibility of uneven distribution of litterfall between row and interow regions, especially for younger plantations that have denser canopy above the row. Hence, it may be necessary to sample both row and inter-row regions of *E. nitens* plantations to estimate rates of litterfall.

The objectives of work reported in this chapter were to estimate the N content of above- and below-ground components, N falling in litter, and subsequently N demand of fertilised and unfertilised trees at 14 *E. nitens* plantations. Although no hypothesis was tested, data gathered were essential for the budget approach reported in chapter 6.

5.2 MATERIALS AND METHODS

5.2.1 Site description

The five sites reported in chapter 2 were sampled along with an additional nine sites. Characteristics of these nine sites are given in Table 5.1. Rates of N application in the fertilised treatments at the sites reported in chapter 2 are supplied in the appendix.

5.2.2 Tissue sampling for N content

Sampling was undertaken from median trees of 5 D^2H (where D is diameter at breast height and H is height) size classes. All live trees in each treatments were ranked in order of D^2H and divided into five size classes of equal numbers of trees from which the median tree was sampled.

Nitrogen concentrations in whole-canopy leaf, representative leaf, bark, branch and wood at the Basils and Boulder sites were estimated at age three years from treatments one and five at the Basils site and one and eleven at the Boulder site, in September 1996 (Treatment five had the largest average D^2H at the Basils site and treatment eleven had the largest average D^2H at the Boulder site, N application rates for these treatments are shown in Table 5.2.

Table 5.2. *Fertiliser treatments at the Basils and Boulder sites.*

Treatment.	N kg ha^{-1} (at planting)	N kg ha^{-1} Year 1	N kg ha^{-1} Year 2
1	0	0	0
5	25	50	100
11	25	200	200

Note, all treatments received 50 kg P ha^{-1} at establishment.

Estimates of whole-canopy N concentration were conducted on a sub-sample of leaves taken from every third leaf pair on every tenth branch of the sample trees. Representative leaf samples were taken from the north-facing portion of the canopy at 70% and 85% canopy height. At 70% canopy height innermost and outermost leaves were sampled. At 85% canopy height, outermost leaves only were sampled. Branches

Table 5.1. Site characteristics of nine additional sites, characteristics the five other sites sampled are shown in Table 2.1

Site	Elevation (m)	Rainfall (mm)	Soil Type *	Parent Material	Year planted	Age fertilised (years)	Fertiliser application rate (kg NH_4^+ ha^{-1})
[A]Hurds	170	1039	Rudosol	Alluvium	1990	3	200
[B]Blue Gum	350	1255	Rudosol	Granite	1990	3	200
[C]Basalt	580	1570	Ferrosol	Basalt	1986	10	200
[D]Chromeys	300	1640	Ferrosol	Basalt	1987	9	200
[E]Wattle	600	1913	Ferrosol	Basalt	1988	5	200
[E]Wages	620	1913	Ferrosol	Basalt	1991	2	200
[E]Rabbit	630	1913	Ferrosol	Basalt	1993	0,1,2	25,200,200
[E]Old Park	640	1913	Ferrosol	Basalt	1990	3	200
[E]Talbot	650	1913	Ferrosol	Basalt	1989	7	200

* *Isbell (1996)*

Weather stations below were the closest to the study sites. Rainfall rates represent long term averages.

[A]*1039 mm rainfall from Weetah weather station, Bureau of Meteorology Hobart*

[B]*1255 mm rainfall from West Ridgley weather station, Bureau of Meteorology Hobart*

[C]*1570 mm rainfall from Hampshire weather station, Bureau of Meteorology Hobart*

[D]*1640 mm rainfall from Takone weather station, Bureau of Meteorology Hobart*

[E]*1913 mm rainfall from Guilford Junction weather station, Bureau of Meteorology Hobart*

containing sample leaves were removed and the first ten fully expanded leaves from the outermost end of the removed branch were sampled at both heights and at 70% canopy height the first ten leaves from the innermost end of the branch were also sampled. Branches from which leaves were sampled for estimation of average canopy N concentration were cut into 3-6 cm lengths, mixed and sub-sampled for analysis for branch N concentration. At 1.3 m stem height a wood core, taken through the stem in a north-south direction, and a bark sample, from the north facing side of the stem was sampled for estimates of stem wood and stem bark N concentration. Similar wood cores and bark samples were also taken in September 1996 at the Basalt, Blue Gum, Chromeys, Hurds, Old Park, Talbot, Wages, and Wattle sites from unfertilised and N fertilised treatments, varying in age from three to 10 years. Fertilised treatments received 200 kg N ha^{-1} (Table 5.1). Sampled tissues were dried at 60°C for 24 hours, ground (<0.05 mm), acid digested, and analysed as below (5.2.5).

5.2.3 Biomass N

Data for the Tim Shea and Nunamara sites, for the biomass of stem wood, stem bark, branch, and leaf, and their N concentrations were obtained from destructive sampling as part of an unpublished study conducted by Cromer RN, Turnbull ST, and LaSala AV, from which standing N mass was estimated to three years of age. Three additional sites are included in the Cromer *et al.* study, Westfield, Middlesex, and Nabowla, from which the above tissue concentrations are available. These biomass and N concentrations were used to estimate N mass of components at the Tim Shea and Nunamara sites. Where biomass was not estimated from destructive sampling it was estimated using regression equations based on diameter and height.

The unpublished Cromer biomass regression equations are of the form:

$$\text{Component biomass (kg)} = a + b(x) + c(x^2) + d(y)$$

where x is the diameter of the trunk at 1.3 m over bark (cm); y is the height (cm) and a, b, c, and d are coefficients.

These equations were used to estimate biomass of leaf, branch, and stem tissues for trees at the Basils, Boulder, Potters, Wattle, Wages, Old Park, Blue Gum, Rabbits, and

Hurds sites from 1994-1996, and for the Talbot, Chromeys, and Basalt sites for 1996. North Forest Products measured diameters and height of trees within these sites. To enable estimates of stem wood and stem bark biomass, stemwood was assumed to consistently be 0.81 of total stem mass. Biomass of trees smaller than 1.30 m in height are not possible using the regression equations, as they have no diameter at breast height. For trees smaller than 1.35 m in height biomass was estimated by assuming a linear increase in biomass with height to average values of 30 trees of 1.35 m in height from the Basils, Boulder, and Rabbits sites. The Hingston and Galbraith (1998) equations were used to estimate biomass of stem-wood, stem-bark, branches, and leaves at the Wattle site for 1995 and for the Wattle, Hurds, Basalt, and Chromeys sites for 1996. The Hingston and Galbraith (1998) equations were of the form;

$$\ln(W) = a + b.\ln(D) + c.\ln(H)$$

where W is the dry weight of the component (kg); D is the diameter at 1.3 m over bark (cm); H is the height (m) and a, b, and c are coefficients.

Biomasses generated from the regression equations and measured tissue N concentrations were used to estimate N mass of tissues. Where tissue N concentrations were not available from destructive sampling they were estimated using a regression of tissue N concentration and age. The regression was formed from *E. nitens* tissue N contents from this study, the unpublished Cromer study and those published in the literature. Because very few data are available for tissue N concentrations of *E. nitens*, data from the literature for *E. globulus* grown in south-east Australia were also included in this regression.

5.2.4 Litter

Litter sampling based on the method of Wilm (1946) using 'fixed' and 'roving' traps has been used in recent times (eg. Attiwill *et al.* 1978). However, Turnbull and Madden (1986) and Turnbull (1982) found no significant quantitative difference between litter collected from fixed and roving traps, or from litter traps placed randomly between trees on rows and in the inter-rows. The canopy at the Basils site was almost at ground level in 1996 and would have interfered with bins or tall litter traps, which would also under-sample that part of the canopy below their rim.

Turnbull and Madden (1986) found no significant difference in litter catches in Tasmanian forests, from bins or ground traps short enough to be placed under the canopy at the Basils site. Hence, for the present study litter was be collected from randomly placed ground traps. Litter traps were placed randomly within the row and inter-row areas of the Basils site at age three years to determine if litter falls evenly on row and inter-row regions.

The ground-traps were 0.10 m high, 0.181 m^2 in area, and constructed from a 0.10 m wide sheet of galvanized iron riveted to form a ring which held fibreglass mesh 7 cm above the ground (Plate 5.1). Litter was collected every 5-8 weeks from the Basils and Basalt sites. At the Basalt site at age 10 years, 12 litter traps were placed randomly within three unfertilised weed controlled plots (four per plot). At the Basils site, 12 litter traps were placed within treatments one and five, four in each of three plots, two placed randomly in the row region and two placed randomly in the region between the rows. Litter from each plot, and from the Basils site, on and between the rows within each plot, was placed into brown paper bags and dried at 60 °C for two days. Tissues were then separated into leaves, bark, and branches and weighed. Sub-samples were taken, ground to a fine powder, dried at 70 °C for 24 h and cooled in a desiccator for N analysis.

5.2.5 N analysis

N was analysed using an acid digestion method based on Lowther (1980). A sub-sample of finely ground (< 0.5 mm) tissue 0.1-0.3 g was placed into 75 mL digestion tubes. Two milliliters of water was used to wash the sample down before the addition of 4 milliliters concentrated H_2SO_4 and mixing. One milliliter H_2O_2 was then added and mixed before the addition of a second milliliter of H_2O_2 and mixing. The oxidising agent H_2O_2 converts all forms of mineral N present to NH_4^+. Samples were placed into a preheated 100 °C block for 10-15 minutes before increasing the temperature to 150 °C for 10 minutes and further increasing the temperature to

Plate 5.1 *Litter trap at the Basalt site.*

200 °C. Prior to increasing the temperature to 360 °C a tear drop stopper was placed onto the tubes. Samples were maintained at this temperature for 30 minutes before cooling to 150 °C and the addition of H_2O_2 drop-wise until the solution turned pale yellow, after which a further six more drops were added. Samples were then reheated to 360 °C and digested for one hour, removed from the block, and cooled, before being made up to 50 mL with water, and mixing on a vortex mixer. Samples were then placed into storage bottles to be analysed for N with Lachat Quickchem 8000 flow injection analyser using Lachat method LACHAT 10-107-06-2E with the same modifications as for total N for soil digests described in chapter 3.

5.2.6 Statistical analysis

Means were compared using LSD where an analysis of variance indicated a significant difference between means ($P \leq 0.05$). Correlations and regressions were determined by standard statistical methods.

5.3 RESULTS

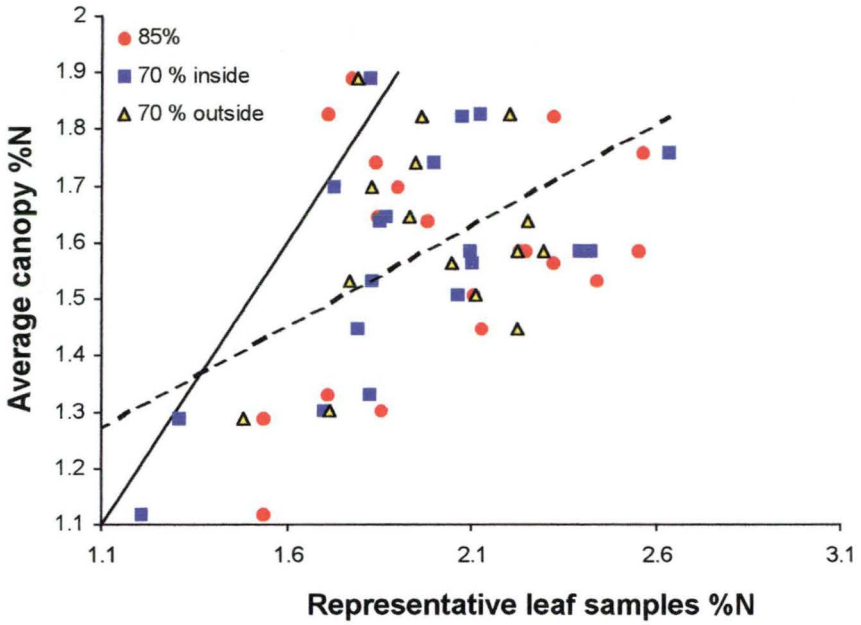
5.3.1 N-concentrations sampled tissues

For stem wood, stem bark, branch, and leaf tissues at the Basils and Boulder sites at age three years, there were no significant differences in mean N concentrations between the fertilised and unfertilised treatments (Table 5.3). At the Basils site, the concentrations of N in leaves, wood, and bark were significantly ($P<0.05$) higher than those at the Boulder site in fertilised and unfertilised treatments. Highest N concentrations were found in leaf tissue (1.42-1.69%), intermediate concentrations for stem bark (0.50-0.75%) and branch tissue (0.38-0.63%), and lowest concentrations for stem wood (0.18-0.26%). Average canopy N concentration ranged from 1.12 to 1.89%. For spot samples, canopy N concentrations ranged from 1.53% to 2.56% at 85% canopy height, 1.21% to 2.63% for inner leaves at 70% canopy height and 1.48% to 2.29% for outer leaves at 70% canopy height. No sample position tested was representative of the canopy. Only representative sampling at 70% inside produced a significant correlation ($P < 0.001$) with average canopy N contents, however the r^2 for this correlation was low (0.37) (Figure 5.1). Hence, estimates of canopy N concentrations require sub-sampling from the entire canopy. This is labour- and time-intensive and hence was not repeated.

Table 5.3. *Tissue N concentrations (%) for leaf, branch, stem bark, and stem wood at the Basils and Boulder sites collected at age three years. Standard deviations are shown in parenthesis (n=3).*

Tissue.	Boulder		Basils	
	Unfertilised	Fertilised	Unfertilised	Fertilised
Leaf	1.42 (0.21)	1.53 (0.22)	1.67 (0.19)	1.69 (0.11)
Bark	0.50 (0.05)	0.51 (0.08)	0.72 (0.08)	0.75 (0.08)
Branch	0.38 (0.10)	0.55 (0.22)	0.57 (0.11)	0.63 (0.10)
Wood	0.21 (0.05)	0.18 (0.02)	0.26 (0.06)	0.25 (0.03)

***Figure 5.1**. Nitrogen concentrations (%N) of representative leaves from sample positions at 85% and 70% canopy height, compared with average canopy %N. At 70% canopy height leaves were taken from the innermost and outermost part of the canopy. At 85% canopy height, outermost leaves only were sampled. Solid line represents 1:1 ratio of average canopy %N : Representative leaf sample %N. Broken line shows the significant ($P < 0.001$) linear regression of 70% inside with average canopy N.*

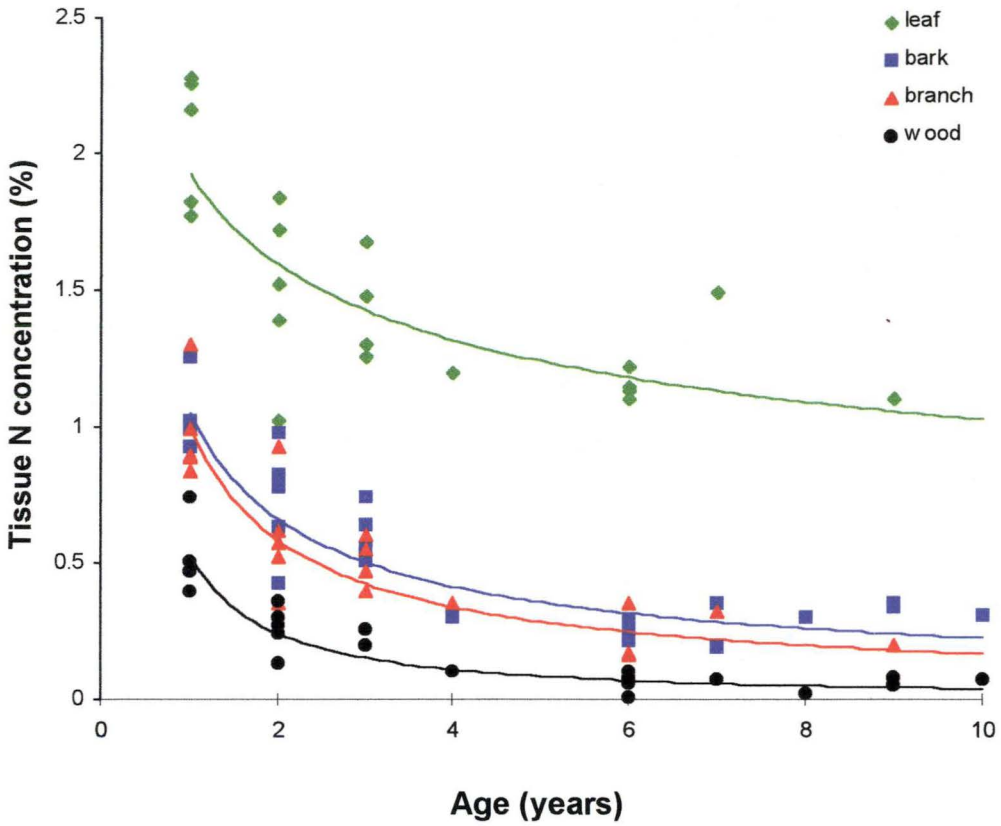


5.3.2 Annual N uptake

Leaf, branch, stem wood, and stem bark N concentrations at sites where there was no destructive sampling of tissues were estimated from regression equations. These equations were constructed by combining N concentration estimates of leaf, branch, stem wood, and stem bark measured by Cromer RN, Turnbull ST, and LaSala AV at five Tasmanian *E. nitens* plantations of ages 1-3 years, from the Basils and Boulder sites destructive sampling of 1996 at age three years, stem wood and stem bark concentrations from destructive sampling at the Basalt, Chromeys, Wattle, Talbot, Blue Gum, and Hurds sights during 1996 when the plantations were of ages 3-10 years, and data from the literature for *E. nitens* and *E. globulus* (Figure 5.2) of ages 4-9 years. Data used to generate Figure 5.2 are shown in the appendix. Equations for leaf, branch, stem bark, and stem wood N concentrations (%), estimated from Figure

5.2 are presented below. Herein, N concentrations predicted using these equations will be referred to as predicted N concentrations.

Figure 5.2. E. nitens *and* E. globulus *leaf, bark, branch, and wood N concentrations with age.*



Data used to construct Figure 5.2 are presented in the appendix

leaves	$= 1.9234.x^{-0.2727}$	$r^2 = 0.62$, n = 20
stem bark	$= 1.0485.x^{-0.6695}$	$r^2 = 0.79$, n = 26
branches	$= 0.9978.x^{-0.7773}$	$r^2 = 0.80$, n = 20
stem wood	$= 0.53.x^{-1.1435}$	$r^2 = 0.75$, n = 24

where x is the plantation age in years.

Trees less than 1.35 m in height were too small for the biomass equations. Thirty trees from the Basils, Boulder, and Rabbits sites of minimum diameter and height for the Cromer equations had stem, branch, and leaf biomasses of 1.05, 0.61 and 0.59 kg,

respectively. Biomass components of smaller trees were assumed to increase in weight linearly with tree height to these values.

Annual above-ground N uptake of fertilised and unfertilised plantations aged 1-3 years at the Tim Shea and Nunamara sites, calculated from destructive sampling for tissue biomass and N concentration (Cromer RN, Turnbull ST, and LaSala AV pers. comm) ranged from 1-2 kg N ha^{-1} $year^{-1}$, 22-39 kg N ha^{-1} $year^{-1}$, and 26-69 kg N ha^{-1} $year^{-1}$ for plantation ages one, two, and three years respectively. These estimates of N uptake were 82-120% of uptake estimated when predicted N concentrations replace those from destructive sampling (Table 5.4).

Standing N mass of fertilised and unfertilised three-year-old *E. nitens* plantations at the Basils and Boulder sites, calculated using the Cromer equations for biomass and destructive sampling for N content ranged from 67-108 kg N ha^{-1}. Differences in N uptake between unfertilised and fertilised treatments within sites were not significant ($P< 0.05$). These estimates of standing N mass were 106-128% of estimates where predicted tissue N contents replace those of destructive sampling (Table 5.4).

Above-ground N content of fertilised and unfertilised plantations aged 1-10 years calculated by multiplying tissue biomasses estimated using the Hingston and Galbraith (1998) and Cromer equations with predicted tissue N concentration ranged from 6-372 kg N ha^{-1} (Table 5.5). These values were used to generate annual N uptake rates for plantations aged 1-8 years, ranging from 5-48 kg N ha^{-1} $year^{-1}$ unfertilised and 5-62 kg N ha^{-1} $year^{-1}$ fertilised. At six of the nine sites, fertilsed trees had significantly greater rates of N uptake than unfertilsed trees (Table 5.6). Average rates of N uptake, estimated from the slope of the regression line in Figure 5.3, for plantations of ages 1-10 years were 20 and 19 kg N ha^{-1} $year^{-1}$ for fertilised and unfertilised plantations respectively. Fertilised treatments remained unfertilised for up to 10 years and all but two of these sites were found to be N sufficient, hence average N uptake rate of fertilised trees is likely to be lower than their potential uptake rate.

Table 5.4. *Above-ground N increment (kg ha^{-1}) of* E. nitens *estimated from destructive sampling for N content at all sites compared with N increment using predicted N contents. Biomass was estimated from destructive sampling at the Tim Shea and Nunamara sites (Cromer* et al. *pers. comm) and predicted using the Cromer equations at the Basils and Boulder sites. Data for "% predicted" is uptake estimated using predicted N concentrations / Uptake estimated using N content from destructive sampling ×100.*

		Unfertilised		Fertilised	
Age	Site	Uptake	% predicted	Uptake	% predicted
0-1	Tim Shea	1	91	2	96
	Nunamara	2	84	2	101
1-2	Tim Shea	22	102	39	117
	Nunamara	28	115	34	101
2-3	Tim Shea	36	114	69	120
	Nunamara	26	82	48	119
0-3	Basils	121	127	138	128
0-3	Boulder	71	106	93	107

Actual tissue concentrations used in Figure 5.2 were 64-131%, 60-160%, 65-149%, and 15-190% of predicted concentrations for leaf, branch, stem-bark, and stem-wood tissues, respectively. Leaf and stem-bark tissue N concentrations varied by not more than 50% of predicted values. Branch and stem-wood tissue N concentrations varied by not more than 90% of predicted values. Varying predicted tissue N concentrations by maximum encountered deviations of ±50% or ±90% could possibly affect estimates of whole tree N uptake by 50-63% (Table 5.7). These are maximum variations of measured tissue N on predicted N biomass; variations likely to be encountered are expected to generally be well within these values.

Table 5.5. *Estimated above-ground N contents (kg ha^{-1}) of* E. nitens *at all sites, except the Tim Shea and Nunamara sites. Biomass was calculated using the Cromer and Hingston and Galbraith (1998) equations where applicable. Predicted N concentrations were used. Standard deviations are in parenthesis.*

	Age	Cromer				Hingston and Galbraith			
Site	(Years)	Unfertilised		Fertilised		Unfertilised		Fertilised	
Potters	1	6	(1)	6	(1)				
	2	11	(1)	11	(1)				
Basils	1	20	(1)	21	(1)				
	2	47	(2)	53	(4)				
	3	95	(4)	108	(10)				
Boulder	1	14	(2)	15	(1)				
	2	33	(1)	38	(6)				
	3	67	(2)	87	(16)				
Rabbits	1	11	(0)	11	(1)				
	2	22	(1)	25	(1)				
	3	40	(3)	44	(2)				
Wages	3	50	(3)	50	(7)				
	4	72	(8)	92	(10)				
	5	91	(16)	123	(13)				
Blue Gum	4	48	(5)	50	(20)				
	5	55	(5)	78	(28)				
	6	69	(9)	113	(31)				
Old Park	4	41	(4)	38	(2)				
	5	54	(4)	62	(3)				
	6	75	(9)	89	(6)				
Hurds	4	63	(12)	68	(7)				
	5	80	(18)	100	(7)				
	6	94	(22)	129	(7)	127	(38)	185	(11)
Wattle	6	108	(6)	115	(16)	162	(6)	169	(31)
	7	113	(6)	128	(14)	177	(31)	196	(31)
	8	125	(6)	159	(13)	186	(12)	254	(25)
Talbot	7	120	(14)						
Chromeys	9	181	(35)			309	(51)		
Basalt	10	215	(20)			372	(36)		

* *tissue concentrations from destructive sampling in 1996 used.*

Table 5.6. *Estimated annual above-ground N uptake (kg ha^{-1} year^{-1}) of* E. nitens *for all sites, except the Tim Shea and Nunamara sites. Biomass was calculated from the Cromer and the Hingston and Galbraith (1998) equations where applicable. Predicted N concentrations were used.*

	Age Period	Cromer equations		Hingston equations	
Site	(Years)	Unfertilised	Fertilised	Unfertilised	Fertilised
Potters	0-1	6	6		
	1-2	5	5		
Basils	0-1	20	21		
	1-2	27 a	31 b		
	2-3	48	56		
Boulder	0-1	14	15		
	1-2	19	23		
	2-3	34	49		
Rabbits	0-1	11	11		
	1-2	11	12		
	2-3	8	11		
Wages	3-4	22 a	41 b		
	4-5	18	32		
Blue Gum	4-5	6 a	28 b		
	5-6	15 a	35 b		
Old Park	4-5	13 a	23 b		
	5-6	20	28		
Hurds	4-5	16 a	32 b		
	5-6	14 a	29 b	30 a	55 b
Wattle	6-7	5 a	13 b	15 a	23 b
	7-8	12 a	31 b	9 a	62 b

Letters denote significant differences between unfertilised and fertilised treatments within sites and years ($P < 0.05$).

Table 5.10. *Estimates of the total amount of N taken up (kg N ha^{-1} $year^{-1}$) into tissues and lost from standing tissues at all sites. Throughfall and stemflow of 10 kg N ha^{-1} $year^{-1}$ are added to all sites (chapter 4).*

Site	Age Years	Above ground N uptake	Below ground N uptake	Litterfall N	Total N uptake
Boulder	2-3	49	19	n.d.	78+litter
Nunamara	2-3	48	19	n.d.	77+litter
Tim Shea	2-3	69	27	n.d.	106+litter
Potters	0-1	6	2	n.d.	18+litter
Basils	2-3	56	22	56	144
[A]Basalt	0-10	22-37	8-15	47	87-109
Wages	3-4	41	16	n.d.	67+litter
Rabbits	1-2	12	5	n.d.	27+litter
Old Park	5-6	28	11	n.d.	49+litter
Wattle	7-8	31	12	n.d.	53+litter
[A]Chromeys	0-9	20-34	8-13	n.d.	38-57+litter
[A]Talbot	0-7	17	7	n.d.	34+litter
Hurds	5-6	29	11	n.d.	50+litter
Blue gum	5-6	35	14	n.d.	59+litter

n.d. *not determined*

[A] *Average annual rates of above-and below-ground N uptake are presented for the given age range.*

6. NITROGEN BUDGETS AND SOIL ANALYSES AS INDICATORS OF NITROGEN DEFICIENCY

6.1 INTRODUCTION

Soil analyses or the determination of an N budget were proposed in earlier chapters as possible indicators of N deficiency in *E. nitens* plantations. Nitrigen fluxes estimated in earlier chapters provide valuable information, helping to contextualise the significance of individual N fluxes, potential N application rates and the timing of these. Efficient measurement of the more significant of these N fluxes or the use of a simpler soil analysis has the potential to predict N deficiency in *E. nitens* plantations.

The formation of an entire N budget is rarely done, due to the many fluxes and difficult measurements involved. Large errors combine to make a budget difficult to close (Bockheim and Leide 1990; Tiedje 1982). For example Binkley *et al.* (1992) found the effects of summing inaccurate estimates of N fluxes in a conifer-alder stand rendered a N budget unclosable. To close an N budget Bormann *et al.* (1977) assumed an N input from fixation, although no symbiotic or asymbiotic N fixers were detected in a cut-stand of the Hubbard Brook experiment. Hence, Bormann *et al.* (1977) concludes, "Measurement of N cycle is no small task. It requires sophisticated techniques, a well designed model for identifying gaps, and years of careful measurement". However, it remains to be tested whether or not a partial N budget based on the most significant and more easily measurable N fluxes will be sufficient to identify N limited forest plantations.

Soil supplies the bulk of N required for tree growth, hence rates of NNM should be included in a partial budget as N supply. Rates of NNM in the 0-10 cm depth ranged from 13 to 188 kg N ha^{-1} $year^{-1}$ from five Tasmanian *E. nitens* plantation soils (Chapter 2). Soil analyses discriminated these sites into two groups, those having NNM greater or less than 40 kg N ha^{-1} $year^{-1}$, and suggest the subsoil mineralises at least twice the N of the top 10 cm (Chapter 3).

Gross N uptake rates required for maximum growth would be ideal for N demand in an N budget. However, maximum growth rates are difficult to ascertain, as this rate is

likely to be site- and management-dependant, involving accumulation of N into tissues as well as subsequent losses. Estimated rates of N uptake into the above-ground biomass ranged from 1-69 kg N ha^{-1} $year^{-1}$, with below-ground N uptake likely to be 0.39 of the above-ground (up to 26 kg N ha^{-1} $year^{-1}$) and N falling in litter of 47-56 kg N ha^{-1} $year^{-1}$ (Chapter 5).

Other N fluxes are small in comparison to NNM and N uptake. For example, in rain (< 8 kg N ha^{-1} $year^{-1}$), N fixation (< 4 kg N ha^{-1} $year^{-1}$), denitrification (not detected in April 1998) and in throughfall and stemflow (< 10 kg N ha^{-1} $year^{-1}$) (Chapter 4). Potentially large N fluxes may occur in leaching. *In situ* leaching (0-10 cm) was estimated at the Basils and Boulder sites as 46 to 50 kg N ha^{-1} $year^{-1}$ and -5 to 23 kg N ha^{-1} $year^{-1}$ respectively (Chapter 2). *In situ* leaching (0-10 cm) within the errors of measurement accounted for NNM in the 0-10 cm depth (70 to 76 kg N ha^{-1} $year^{-1}$ and 17 to 23 kg N ha^{-1} $year^{-1}$ for the Basils and Boulder sites respectively), representing a large loss of mineral N from surface soil. However, although leaching is significant in surface soils within the study sites, with deep penetrating roots and an anion exchange capacity at depth, leaching beyond the rooting zone, and hence leaching losses from the studied system may be small (chapter 2).

Estimating leaching in the structured clay-loam soils of the study sites using techniques such as the porous ceramic cups is not recommended (Addiscott 1996). Lysimeters provide a better but more labour intensive approach to estimate N leaching. However, potential errors are encountered with the creation of an air-water interface between the soil and the collector. The most suitable method to measure leaching is likely to be via a model, such as APSIM (McCrown *et al.* 1996) or LEACHN (Ramos and Carbonell 1991). However, considerable effort is required to collect data for the model parameters.

Weeds can compete strongly for water (Clinton and Mead 1994a; Eissenstat and Mitchell 1983) and N (Clinton and Mead 1994b; Smethurst and Nambiar 1989b; Neary *et al.* 1990; Nambiar 1990; Eastham and Rose 1990) due to high root length densities (Nambiar 1990, Eastham and Rose 1990). Weed N uptake can be large, for example weeds growing in a *P. taeda* plantation immobilised up to 55 kg N ha^{-1} $year^{-1}$

(Neary *et al.* 1990), and in a *P. radiata* plantation 69-171 kg N ha^{-1} $year^{-1}$ (Woods *et al.* 1992). Hence, weeds can reduce soil mineral N concentrations, tree N uptake, and growth (Smethurst and Nambiar 1989a; Smethurst and Turvey 1986; Neary *et al.* 1990), and therefore herbicide was applied to control weeds at the study sites.

Estimates of *in situ* NNM (0-10 cm depth) had standard deviations of up to 200% (Table 2.2) and were not significantly different to estimates of *in situ* nitrification, leaching (0-10 cm depth) (Chapter 2), and uptake into above- and below-ground biomass (this chapter). Soil analyses could be used to divide sites only into those having NNM greater or less than 40 kg N ha^{-1} $year^{-1}$, for 0-10 cm depths. Estimates of N uptake into the standing biomass are based on assumptions of validity of biomass regression equations and assumed tissue concentrations for the study sites. Considering the large errors and potential errors involved with measurement of NNM and uptake the smaller N fluxes mentioned above were considered to be insignificant and not considered in the budgeting approach, and the effort required to estimate N leaching was, for this study, not justified.

Rates of NNM have been correlated to forest productivity in the literature (Chapter 2). Hence, soil analyses correlated with NNM may also be correlated with growth responses to N fertilisation of *E. nitens* plantations. The soil analyses anaerobically mineralisable N, hot KCl extractable N, total N, total C, and total P were able to divide the five sites described in chapter 2 as described above and may correlate to growth responses of *E. nitens* to N fertilisation. Estimates of concentrations of soil mineral N concentrations may indicate N sufficiency at the time of sampling (Chapter 3). Hence, estimates of concentrations of ammonium and nitrate in soil solution and cold KCl extract may, for forest soils, provide an estimate of N sufficiency of soil at the time of sampling, as suggested by Rice and Havlin (1994). As soil solution N and cold KCl extractable N concentrations fall, responses to N fertiliser may develop.

The objective of work reported in this chapter was to evaluate budget and soil analysis methods as predictors of when N fertiliser was required to maximise growth of *E. nitens* plantations. Soil analyses were used to estimate NNM at the nine sites mentioned in chapter 5.

6.2 MATERIALS AND METHODS

6.2.1 Fertiliser responses

Growth responses were calculated from measurements of diameter at breast height (1.3 m stem height) at the Basils, Boulder, Potters, Rabbits, Wattle, Wages, Old Park, Blue Gum, and Hurds sites from 1994-1998 and for the Talbot, Chromeys, and Basalt sites for 1996-1998 by G Holz, North Forest Products. R Cromer calculated responses from diameters at breast height at the Tim Shea and Nunamara sites for years 1993-1996.

6.2.2 Net N mineralisation in surface soils

Nine sites described in chapter 5 (Table 5.1) were sampled, as for initial soil as described in 2.2.3, for soil analysis in September 1997. These sites had similar cultivation and planting regimes as the five sites from chapter 2, except they all received 21.6 kg N and 24.0 kg P per ha^{-1} at planting. Soil analyses, hot KCl extractable N, anaerobically mineralisable N, total N, total P, and total C were used to separate these sites into those having NNM greater or less than 40 kg N ha^{-1} $year^{-1}$.

6.2.3 Weed growth

Weed biomass and N uptake were not estimated at any site. Weeds did not require controlling at the Basils, Boulder, and Nunamara sites where bare soil was visually estimated to be 90% or greater. At the Potters site, pasture grass and weed growth was considered to be a problem in October 1996 and March 1997 with rapid growth resulting in up to 100% ground cover. As a result the entire site was sprayed with herbicide in January 1997 and the unfertilised treatments were also sprayed in March 1997. At the Tim Shea site, herbaceous weed growth was considered a problem in February and December 1996 with rapid weed growth resulting in up to 80% ground cover. Herbicide was applied to the control plots in April and June of 1996 and in January 1997. In March 1997 weeds in sprayed and unsprayed regions had died resulting in weed coverage falling below 20%.

Smethurst and Nambiar (1989b) found weed cover of 20% in a *P. radiata* plantation reduced soil mineral N levels, reducing the supply of mineral N to trees. Hence, weed competition may have reduced soil mineral N levels and slowed tree growth at the

Tim Shea and Potters sites. The effect of weeds on *Eucalypt* growth decreases after age four years (Holz pers. comm.). There was no significant difference in diameter increment at breast height between sites with and without weed control over a two year period at the Basalt site and over a three year period at the Old Park, Blue Gum, and Hurds sites when weed control was initiated at ages 3-10 years (Holz pers. comm.). Hence the effects of weeds on growth of trees older than three years is expected to be small.

6.2.4 Budget approach

Partial budgets were constructed with N input as NNM and output as uptake into biomass. For partial budget 'a', NNM at 0-10 cm depth was tripled to allow for subsoil NNM (Chapter 3). Nitrogen uptake was presumed to be into above-ground and below-ground biomass and into litter. For partial budget 'b', NNM was for 0-10 cm depth only and uptake was presumed to be into above-ground biomass only. A response was predicted when NNM was less than or equal to N uptake of fertilised trees.

6.2.5 Soil analysis approach

Soil analyses for correlation with growth response of *E. nitens* plantations to N fertiliser additions were the same soil analyses as those for correlation with annual NNM described in chapter 2. Soil analyses were conducted on sieved initial soils as described in 2.2.3 from the nine sites listed in Table 5.1, and sampled as described in chapters 2 and 3. Estimates of total C are not possible on granitic soils using Wang *et al.* (1996b) regression equations. Hence, the granitic soil at the Blue Gum site does not have an estimate of total C or C:N ratio.

6.2.6 Statistical analysis

Means were compared using LSD, where an ANOVA showed a significant difference between means ($P \leq 0.05$). Correlations and regressions were determined by standard statistical methods.

6.3 RESULTS

6.3.1 Fertiliser responses

Table 6.1 shows when responses were recorded within the study sites. The first year that fertilised trees had significantly greater diameters at breast height than unfertilised trees at the Boulder, Nunamara, Tim Shea, and Rabbits sites, was year one, two, three, and four respectively. No response was observed for the Potters site up to age three years or for the Basils site up to age four years. Significant ($P<0.05$) relative responses in diameter increment at breast height (diameter increment of fertilised trees / diameter increment of unfertilised trees) for two years growth after application of N fertiliser were 171, 142, 132, 127 and 121% at the Blue Gum, Wattle, Wages, Hurds, and Old Park sites respectively. For the Chromeys, Basalt, and Talbot sites, non-significant ($P<0.05$) relative responses in diameter increment at breast height for two years growth after application of N fertiliser were 102, 99, and 116% respectively (Table 6.2). However, in 1999, three years after fertiliser application there was a significant response in diameter growth at breast height at the Talbot site. (Holz pers. comm.). Images of the responding four-year-old Tim Shea site, the seven-year-old Old Park site, the not yet significant response at the eight-year-old Talbot site (a significant response occurred two years after the time of the photo), and the non significant response at the 2.5 year-old Basils site and the nine-year-old Chromeys site are shown on Plates 6.1, 6.2, 6.3, 6.4, and 6.5, respectively.

6.3.2 Net N mineralisation indices in surface soils

Anaerobically mineralisable N ranged from 18 μg N g soil^{-1} at the Blue Gum site to 130 μg N g soil^{-1} at the Basalt site. Hot KCl extractable varied from 9 μg N g soil^{-1} at the Blue Gum site to 110 μg N g soil^{-1} at the Basalt site. Total N varied from 0.10% at the Blue Gum site to 0.57% at the Basalt site. Total P varied from 0.00% at the Blue Gum site to 0.18% at the Rabbits site. LOI varied from 2.4% at the Blue Gum site, to

Table 6.1. *Planting year, age fertilised and fertiliser responses at all 14 sites (P<0.05).*

	Planted (year)	Age fertilised (Years)	Response, year after initial fertiliser application				
Chapter 2 sites			1	2	3	4	5
Boulder	93	0, 1, 2	R				
Basils	93	0, 1, 2	n.s.	n.s.	n.s.	n.s.	n.s
Nunamara	93	1, 2, 3	n.s.	R			
Tim Shea	93	1, 2, 3	n.s.	n.s.	R		
Potters	95	0	n.s.	n.s.	n.s.	n.s.	n.d.
Chapter 5 sites							
Rabbits	93	0, 1, 2	n.s.	n.s.	n.s.	R	
Wages	91	3	R				
Blue Gum	90	4	R				
Hurds	90	4	R				
Old Park	90	4	R				
Talbot	89	7	n.s.	n.s.	R		
Wattle	88	6	R				
Chromeys	87	9	n.s.	n.s.	n.s.	n.d.	
Basalt	86	10	n.s.	n.s.	n.s.	n.d.	

R = *Significant response (P<0.05)*

n.s. *indicates not significant*

n.d. *indicates not determined*

Table 6.2. *Soil analysis values and year of response or % responses in growth of* E. nitens *to N fertiliser for all sites. % Responses are diameter at breast height increment of N fertilised trees over control trees for two years growth after N application. Where there were significant differences in soil analysis values with sample period parenthesis enclose average values observed in September 1996 and within 8 days of September 1997 respectively.*

Site	Total N (%)	Total P (%)	Total C (%)	A.M.N μg N g soil^{-1} week^{-1}	hot KCl μg N g soil^{-1}	Year Planted (Years)	Age Fertilised (Year)	Age Responded	Response
Chapter 2 sites									Year of response
Basils	0.74	0.32	13.3	155 (129-224)	185 (179-188)	1993	0,1,2	n.s	N.R. to age 4
Potters	0.65	0.41	9.1	113 (141-132)	164 (168-173)	1993	0	n.s	N.R. to age 3
Tim Shea	0.33	0.08	6.1	101 (72-149)	62 (56-108)	1993	1,2,3	3	3
Nunamara	0.22	0.08	3.4	48 (27-54)	10 (7-11)	1993	1,2,3	2	2
Boulder	0.27	0.05	6.7	62 (72-60)	12 (9-16)	1995	0,1,2	1	1
Chapter 5 sites									
Rabbits	0.51	0.18	8.8	100	66	1993	0,1,2	4	4
									% response
Basalt	0.57	0.14	10.8	130	110	1986	10	n.s	99
Chromeys	0.49	0.17	8.5	127	97	1987	9	n.s	102
Talbot	0.38	0.15	7.1	68	64	1989	7	10	116
Old Park	0.50	0.15	10.0	80	71	1990	3	4	121
Wages	0.51	0.17	8.8	91	97	1991	2	3	127
Hurds	0.18	0.03	3.9	33	26	1990	3	4	132
Wattle	0.50	0.18	8.5	99	85	1988	5	6	142
Blue Gum	0.10	0.00		18	9	1990	3	4	171

n.s. *indicates not significant* A.M.N = *Anaerobically Mineralisable N* *N.R. indicates no response*

Plate 6.1 The *Tim Shea site in January 1997, age 4 years. The blue box is on the boundary of fertilised and unfertilised treatments with larger fertilised trees behind the box.*

Plate 6.2 The *Old Park site in December 1997, at age 7 years. The blue box is on the boundary of fertilised and unfertilised treatments with larger fertilised trees behind the box. Note the low coverage of weeds.*

Plate 6.3 The *Talbot site in November 1997, at age 8 years. Trees in the foreground are unfertilised. Trees with larger canopies (4 stems in) are fertilised.*

Plate 6.5 *The Chromeys site December 1997, at age 9 years. Note low coverage of weeds present and litter coverage comprising mostly leaves.*

Plate 6.4 *The Basils site in January 1996, at age 2.5 years, prior to the onset of litterfall. Note the low coverage of weeds.*

11.2% at the Basalt site. Not including the Blue Gum site total C ranged from 3.9% at the Hurds site, to 11.2% at the Basalt site. C:N varied from 14.9 at the Hurds site to 20.8 at the Old Park site (Table 6.2). Correlations between total N, total C, total P, anaerobically mineralisable N, and hot KCl extractable N at the nine sites from chapter 5, are shown in Table 6.3 and with all 14 sites in Table 6.4.

Table 6.3. *Coefficient's of determination (r^2) for linear relationships between N mineralisation and soil tests at the nine sites described in chapter 5. (n=3 per site) (For all relationships P<<0.001).*

	1	2	3	4
1. Total N				
2. Total C	0.93			
3. A.M.N.	0.75	0.68		
4. Hot KCl	0.80	0.72	0.74	
5. Total P	0.82	0.71	0.60	0.69

A.M.N = *Anaerobically Mineralisable Nitrogen*

Table 6.4. *Coefficient's of determination (r^2) for linear relationships between soil tests at the 14 sites (five sites described in chapter 2 and nine described in chapter 5) (For all relationships P<<0.001).*

	1	2	3	4
1. Total N				
2. Total C	0.85			
3. A.M.N	0.73	0.70		
4. Hot KCl	0.86	0.67	0.68	
5. Total P	0.73	0.46	0.45	0.82

n=3 for all sites but for Potters site where n=5

A.M.N = *Anaerobically Mineralisable Nitrogen*

Sites examined in chapter 2 were divided into those having rates of NNM greater or less than 40 kg N ha^{-1} $year^{-1}$, where sites with NNM > 40 kg N ha^{-1} $year^{-1}$ had total C > 8%, total N > 0.4%, total P > 0.2% and hot KCl extractable N > 100 μg N g^{-1} soil and those with NNM < 40 kg N ha^{-1} $year^{-1}$ with lower equivalent soil analyses values. Using hot KCl extractable N as an indicator of NNM, the Basalt and Wages sites were

identified as having NNM > 40 kg N ha^{-1} $year^{-1}$. When total N and total C were used as indicators of NNM the Rabbits, Old Park, Wattle, and Chromeys sites were also assessed as having NNM > 40 kg N ha^{-1} $year^{-1}$, and the Talbot site was included when total P was used

6.3.3 Budget approach

A response was predicted when uptake was greater or equal to NNM of unfertilised trees. Of the five sites where NNM was measured *in situ,* the more complex partial budget 'a', which included estimates of subsoil NNM (below 10 cm), uptake into above- and below-ground biomass and N demand of litter losses, predicted responses at the Boulder, Tim Shea and Nunamara sites. However, the year of predicted response was one year after the actual response at the Nunamara site and two years after the actual response at the Boulder site (Table 6.5). The simpler partial budget 'b', which included only NNM of the top 10 cm and uptake into above-ground biomass only accurately predicted observed responses to N in all years where data was available (Table 6.6). When soil analyses were used to predict rates of NNM, the observed response was predicted at only one of the five sites. Dividing rates of NNM into greater or less than 40 kg N ha^{-1} $year^{-1}$ was insufficient to predict responses at the remaining four sites. Of the 14 study sites, partial budget 'b' using soil analyses to predict NNM, accurately predicted observed responses at 3-5 sites, inaccurately predicted observed responses at 3-5 sites and was insufficient to predict a response at 5-10 study sites, depending on the soil analysis used (Table 6.7).

6.3.4 Soil analysis approach

Soil analyses and year of response at the Boulder, Nunamara, Tim Shea, Basils, Potters, and Rabbits sites and % response of the Wages, Blue Gum, Old Park, Hurds, and Wattle sites are shown in Table 6.2. Total P, total C, and hot KCl extractable N were used to form two groups of sites, those that responded to added N by age three years and those that did not respond to added N by age three years. Responding sites had total P less than 0.18%, total C less than 8.8%, total N less than 0.4% and hot KCl extractable N less than 56 μg N g $soil^{-1}$. Anaerobically mineralisable N results were used to separate sites into those that did or did not respond to added N by age three

Table 6.5. *Partial N budget 'a' for the five sites described in chapter 2. NNM is* in situ *(0-10 cm)(Chapter 2), trebled to account for the subsoil (Chapter 3). Uptake estimate included N taken up into above-ground biomass, below-ground biomass and in litter (assumed to be 56 kg N ha^{-1} year^{-1} for plantation ages 2-3 years and older). Ages of initial response are shaded.*

Site	Age (Years)	Unfertilised Uptake [A]	NNM (0-10 cm ×3) [A]	Balance	Observed Responses Predicted
Potters	0-1	8	n.d.		n.d.
	1-2	7	564	557	Yes
Basils	0-1	28	n.d.		n.d.
	1-2	38	273	235	Yes
	2-3	123	210	87	Yes
Boulder	0-1	19	n.d.		n.d.
	1-2	26	54	28	No
	2-3	103	72	-31	Yes
Tim Shea	0-1	9	n.d.		n.d.
	1-2	31	162	131	Yes
	2-3	106	39	-67	Yes
Nunamara	0-1	3	n.d.		n.d.
	1-2	39	69	30	No
	2-3	92	69	-23	Yes

[A] (kg N ha^{-1} year^{-1})

n.d. *indicates not determined*

years. Responding sites had anaerobically mineralisable N of less than 100 μg g soil^{-1} week^{-1} in September. Total N values were used to separate sites into those that responded to added N by age three years (total N < 0.27%), those that responded at age three years (total N 0.28%-0.51%) and those that did not respond by age three years (total N > 0.51%) (Table 6.1). Soil analysis, total C, total P, Total N, hot KCl extractable N and anaerobically mineralisable N, correlated with % response at the Basalt, Blue Gum, Chromeys, Old Park, Hurds, Talbot, Wages, and Wattle sites with r^2 of 0.12 (P = 0.31), 0.41 (P =.09), 0.52 (P= 0.04) , 0.58 (P = 0.03) and 0.61 (P = 0.02) respectively. Anaerobically mineralisable N was able to separate sites fertilised at ages 3-10 years into those that did and did not respond within three years after fertiliser application, where responding sites had anaerobically mineralisable N less than 127 μg N g^{-1} soil week^{-1} and non responding sites had equivalent or greater values. Figure 6.1 shows the correlation of total N (the soil analyses of greatest utility in separating sites by initial year of fertiliser response) and anaerobically

mineralisable N (the soil analysis able to separate sites fertilised at ages 3-10 years into those that did and did not respond three years after fertiliser application) with relative responses of sites fertilised at ages 3-10 years.

Table 6.6. *Partial N budget 'b' for the five sites from chapter 2. NNM is* in situ *(0-10 cm) (Chapter 2). Uptake is into the above-ground only. Ages of initial response are shaded.*

Site	Age (Years)	Unfertilised Uptake [A]	NNM (0-10 cm) [A]	Balance	Observed Responses Predicted
Potters	0-1	6	n.d.		n.d.
	1-2	5	188	183	Yes
Basils	0-1	20	n.d.		n.d.
	1-2	27	91	64	Yes
	2-3	48	70	22	Yes
Boulder	0-1	14	n.d.		n.d.
	1-2	19	18	-1	Yes
	2-3	34	24	-10	Yes
Tim Shea	0-1	1	n.d.		n.d.
	1-2	22	54	32	Yes
	2-3	36	13	-23	Yes
Nunamara	0-1	2	n.d.		n.d.
	1-2	28	23	-5	Yes
	2-3	26	23	-3	Yes

[A] (kg N ha^{-1} $year^{-1}$)

n.d. *indicates not determined*

Soil solution and cold KCl extractable ammonium and nitrate concentrations for the five sites described in chapter 2 are shown in Figure 6.2 and 6.3 respectively. Soil solution and cold KCl extractable ammonium and nitrate for the nine sites described in chapter 5, sampled in September 1997, are shown in Figure 6.4. Soil solution extracts from the Hurds site were mishandled. The Boulder, Blue Gum, Hurds, Nunamara, Old Park, Talbot, Tim Shea, Wages and Wattle sites responded to N fertiliser and all had soil solution and cold KCl nitrate values below 0.1 mM and 1 μg N g^{-1} soil respectively. The Basalt, Basils, Chromeys and Potters sites did not respond

Table 6.7. *Partial N budget 'b' for all 14 sites. NNM was estimated from soil analyses (Chapter 3). Uptake is into the above-ground only. Age of initial response are shaded*

Site	Age (Years)	Age Fertilised	Unertilised Uptake	NNM (0-10 cm)	Observed Responses Predicted		
					a	b	c
Potters	0-1	0	6	>40 a,b,c	Yes	Yes	Yes
	1-2		5				
Basils	0-1	0,1,2	20	>40 a,b,c	?	?	?
	1-2		27				
	2-3		48				
Boulder	0-1	0,1,2	14	<40 a,b,c	?	?	?
	1-2		19				
	2-3		34				
Tim Shea	0-1	1,2,3	1	<40 a,b,c	?	?	?
	1-2		22				
	2-3		36				
Nunamara	0-1	1,2,3	2	<40 a,b,c	?	?	?
	1-2		28				
	2-3		26				
Rabbits	0-1	0,1,2,3	11	<40 a, >40 b,c	?	Yes	Yes
	1-2		11				
	2-3		8				
Wages	3-4	3	22	>40 a,b,c	No	No	No
	4-5		18				
Blue Gum	4-5	4	6	<40 a,b,c	?	?	?
	5-6		15				
Old Park	4-5	4	13	<40 a, >40 b,c	?	No	No
	5-6		20				
Hurds	4-5	4	16	<40 a,b,c	?	?	?
	5-6		14				
Wattle	6-7	6	5	<40 a, >40 b,c	?	No	No
	7-8		12				
Talbot	0-7	7	17 [z]	<40 a,b, >40 b	?	Yes	Yes
Chromeys	0-9	9	34 [z]	<40 a, >40 b,c	?	Yes	Yes
Basalt	0-10	10	37 [z]	>40 a,b,c	Yes	Yes	Yes
% predicted					21	36	36

[z] *Average annual above ground N uptake for years indicated.*
a NNM >40 kg N ha^{-1} $year^{-1}$ (0-10 cm) *based on hot KCl.*
b NNM >40 kg N ha^{-1} $year^{-1}$ (0-10 cm) *based on total N and total C*
c NNM >40 kg N ha^{-1} $year^{-1}$ (0-10 cm) *based on total P*
? *indicates unable to predict*

Figure 6.1. *Correlation and 95% prediction intervals of total N (r^2=0.52) and Anaerobically Mineralisable N (AMN) (r^2=0.61), with relative response of*

sites fertilised at ages 3-10 years.

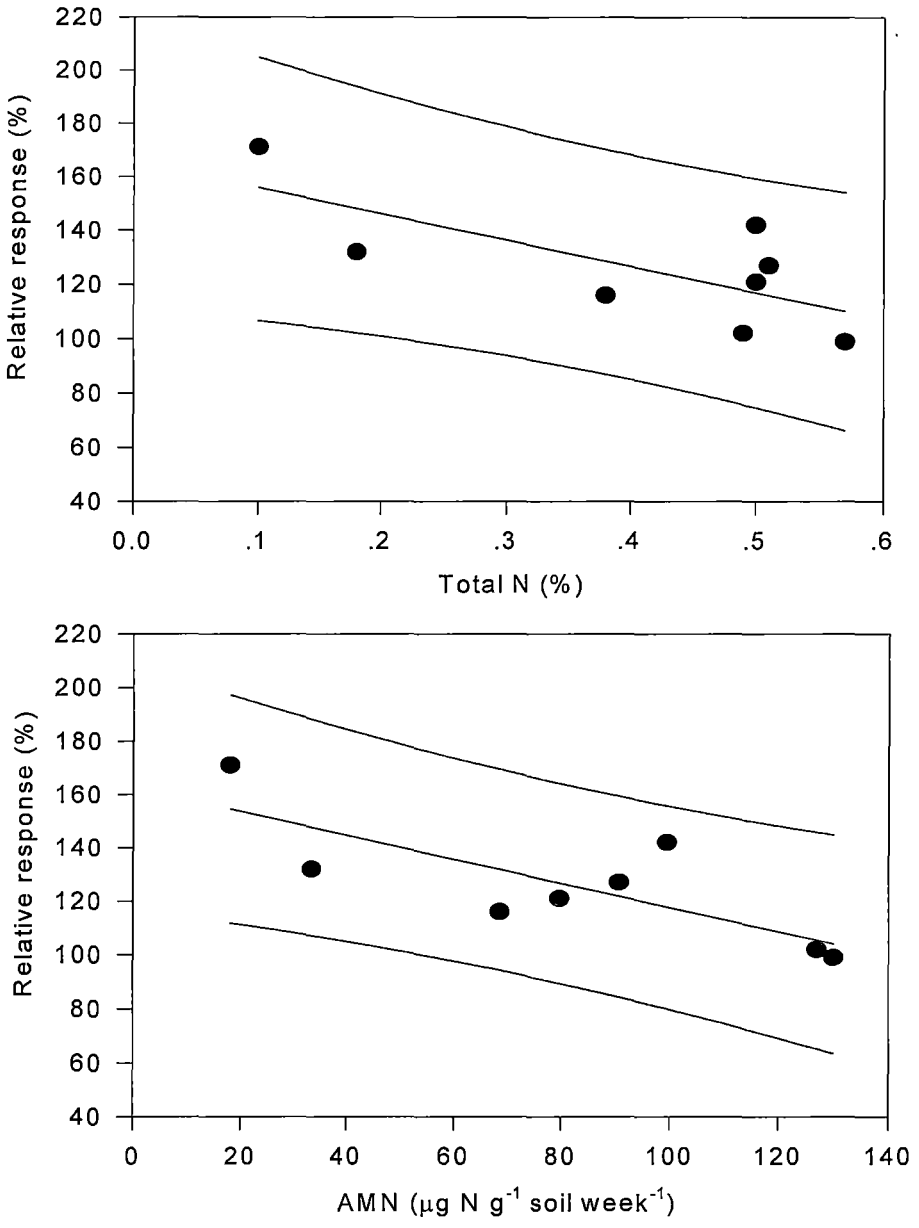
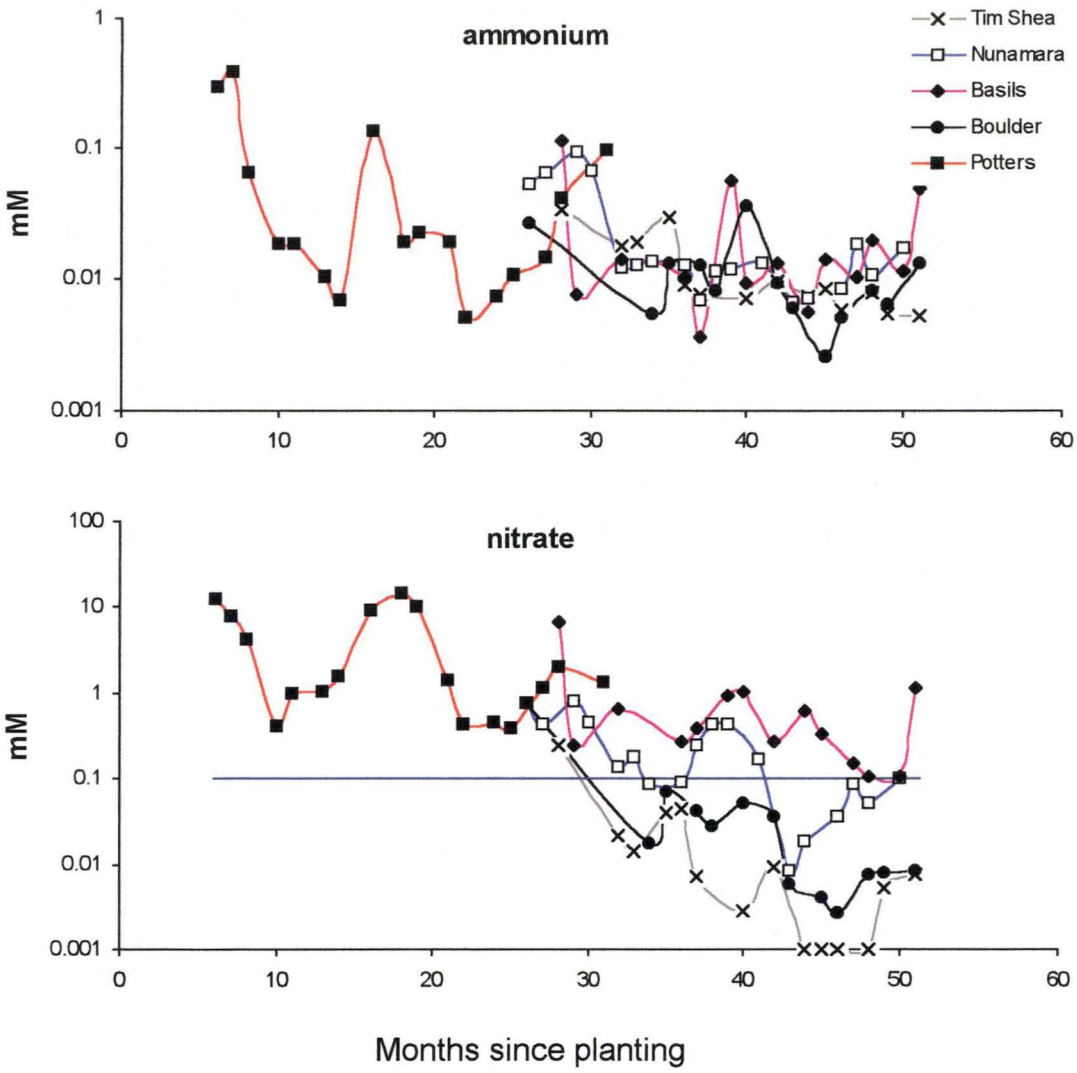


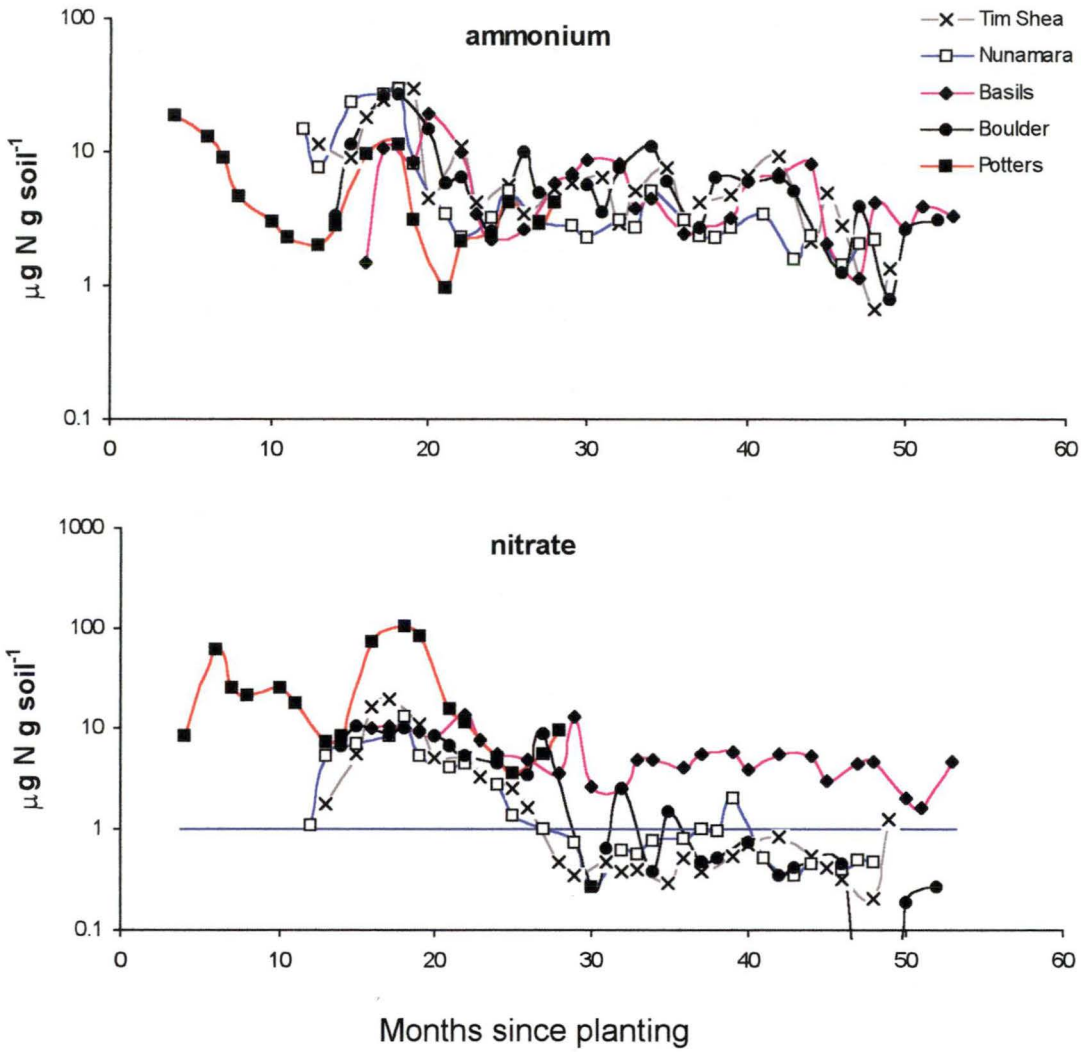
Figure 6.2. *Soil solution extractable N with time at the Boulder, Nunamara, Tim Shea, Basils, and Potters sites.*



to N fertiliser and had some soil solution values above and 0.1 mM and cold KCl extractable values and had some soil solution values above and 0.1 mM and cold KCl extractable values above 1 μg N g^{-1}. For the Boulder, Nunamara and Tim Shea sites the onset of fertiliser responses did not coincide with the fall in mineral nitrate below soil 0.1 mM or 1 μg N g^{-1} soil. However, concentrations of NO_3^- remained below these critical levels for significant periods beyond age 30 months at these sites. Soil solution and cold KCl extractable ammonium concentrations were not able to separate sites by fertiliser response. The responding Boulder, Nunamara and Tim Shea sites had similar ammonium concentrations to the non responding Basils and Potters sites (Figures 6.2

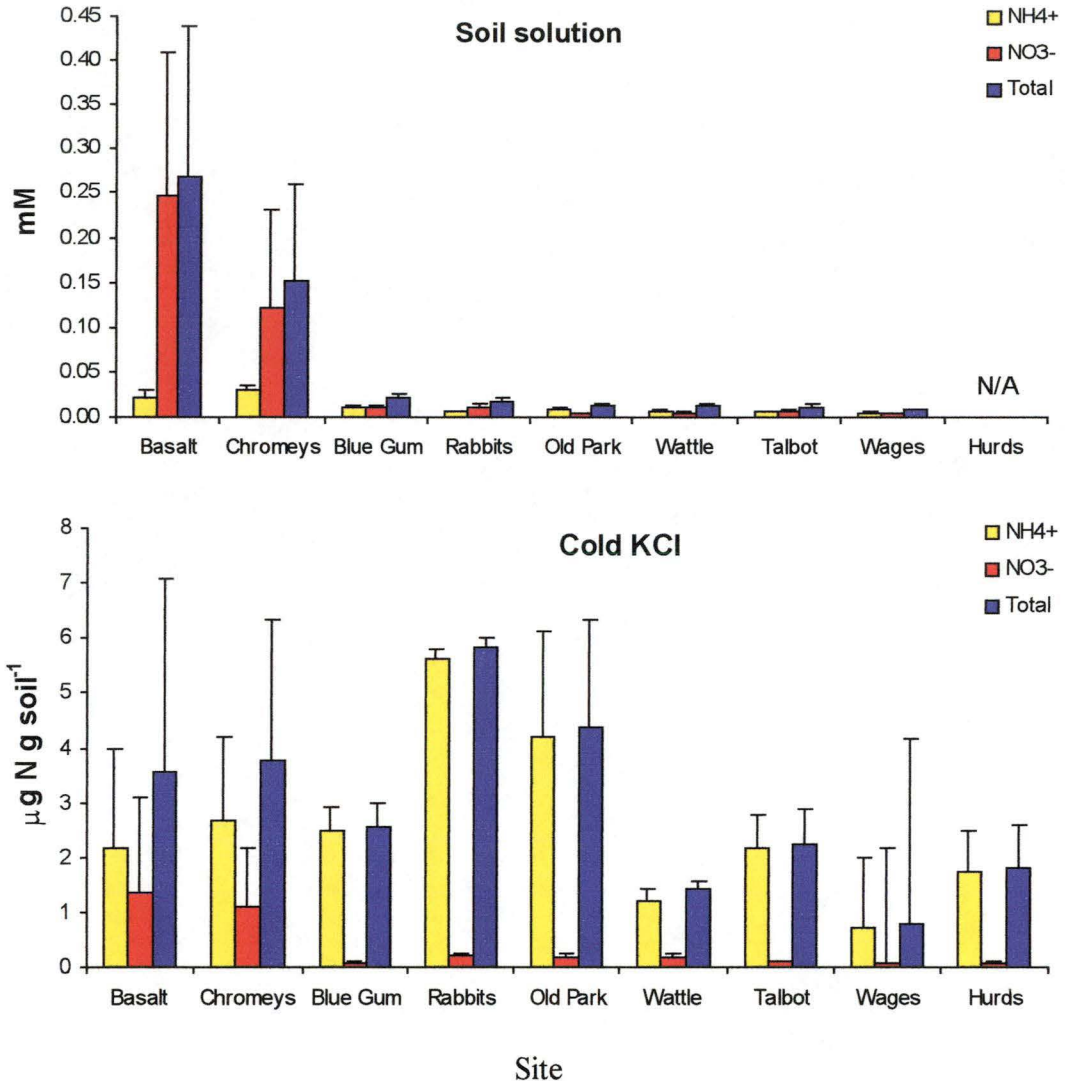
and 6.3). Similarly for the nine sites from chapter 5, non responding Basalt and Chromeys sites had similar ammonium concentrations to the responding sites (Figure 6.4).

Figure 6.3. *Cold KCl extractable N with time at the Boulder, Nunamara, Tim Shea, Basils, and Potters sites.*



Data for the Boulder, Nunamara, Tim Shea, and Basils sites before months 24, 24, 25, and 26 respectively are from Wang et al. (1998).

Figure 6.4. *Soil solution N and Cold KCl extractable N in unfertilised soil at the sites fertilised at ages 3-10 years. Bars show standard deviations.*



6.4 DISCUSSION

For the five sites with measures of *in situ* NNM the simpler partial budget, accounting only for NNM in the top 10 cm and N uptake into the above-ground biomass, accurately predicted fertiliser responses at all five sites. The more complex budget, accounting for surface and subsoil NNM, uptake demand of above-ground biomass, below- ground biomass and losses to litter identified sites that responded by age three years, however two of these sites responded earlier (1-2 years) than this model predicted. When soil analyses were used to predict NNM the ability to predict fertiliser responses reduced. Fertiliser responses were accurately predicted at a maximum of five of the 14 study sites, including only one of the five sites with measures of *in situ* NNM. The ability of soil analyses to divide sites only into those of greater or less than 40 kg N ha^{-1} $year^{-1}$ was too inaccurate to predict responses at 5-10 of the 14 study sites (depending on the soil analysis used). Hence, to construct a usefully predictive partial budget, *in situ* NNM or a soil analysis well correlated to the encountered range of NNM is required.

In an unfertilised ecosystem N budget, the N taken up by vegetation should not exceed the amount supplied by NNM in the soil. Uptake of N into above- and below-ground tissues described in chapter 5 was not significantly greater ($P < 0.05$) than annual NNM (0-10 cm) described in chapter 2 for 1995/96 at the Basils, Nunamara and Potters sites, but was significantly greater at the Boulder and Tim Shea sites. From results reported in chapter 3, all indices of NNM indicated that the subsoil mineralised at least twice the amount of the top 10 cm. If annual NNM rates from 0-10 cm depths are only doubled to account for NNM potential below 10 cm, then at no site was annual uptake significantly greater than annual NNM.

Sites fertilised at planting were separated by year of initial fertiliser response with greatest utility by values of total N, which separated sites into those that respond before age three years (n=2), at age three years (n=1) and those that did not respond by age three years (n=3). For the five sites described in chapter 2 there were no significant differences in total N for soil sampled at six month intervals over a two year period, and annual NNM was only 0.5-3.4% of total N (chapter 3). Hence values of total N are temporally stable and samples can be taken at any time of year.

Soil analyses total C, total P, total N, hot KCl extractable N and anaerobically mineralisable N correlated with % response in diameter growth at the Wages, Blue Gum, Old Park, Hurds, Wattle, Basalt, Chromeys, and Talbot sites with r^2 of 0.12, 0.41, 0.52, 0.58 and 0.61 respectively. Only values of anaerobically mineralisable N were able to separate the study sites into those that responded and those that did not respond to added N three years after N application, where responding sites had anaerobically mineralisable N less than 127 $\mu g\ N\ g^{-1}$ soil $week^{-1}$. This finding is similar to that of Powers (1980) where *P. ponderosa* grown on volcanic, metavolcanic and metasedimentary soils was likely to respond with anaerobically mineralisable N below 12 ppm. The large temporal variations encountered with this soil analysis (chapter 3) require explaining before anaerobically mineralisable N can confidently be used to predict fertiliser responses. There was a significant ($P < 0.05$) response (relative response) to N fertiliser applied to the Blue Gum, Hurds, Old Park, Wages, and Wattle sites of 121-171%. The Basalt and Chromeys sites did not have significant ($P < 0.05$) responses to added N yet values of total P at the Basalt site and values of total N, total P and total C at the Chromeys site were lower than values for 3-4 of the six responding sites. However the responding Blue Gum and Hurds sites had the lowest soil analysis values for all soil analyses. Hence sites of total N $\leq$ 0.18%, total P $\leq$ 0.03% and total C $\leq$ 3.9% may identify only very poor sites that will respond to added N when fertilised at ages 3-10 years. Values of hot KCl extractable N were the same at the non-responding Chromeys site and the responding Wages site. Hence, if responsive sites were identified as having hot KCl extractable N values of less than 97 $\mu g\ N\ g^{-1}$ soil it would accurately identify five of the six (83%) responsive sites. The simplicity and temporal stability of this soil analysis is attractive, possibly inviting its use to identify the majority of responsive sites.

Ammonium concentrations with either cold KCl extractable N or soil solution N were not able to indicate the onset of N deficiency. The non-responding Potters and Basils sites had similar NH_4^+ concentrations as the responding Boulder, Nunamara, and Tim Shea sites (Figures 6.1 and 6.2). Concentrations of soil solution and cold KCl extractable NO_3^- at the Boulder, Nunamara, and Tim Shea sites did drop below concentrations at the Potters and Basils sites, but this did not coincide with the onset

of fertiliser responses. The Tim Shea, Nunamara and Boulder sites responded at age three years, two years and one year respectively. Soil solution NO_3^- values dropped below 1 mM after 33, 35, and 35 months for the Tim Shea, Nunamara, and Boulder sites respectively. Cold KCl extractable NO_3^- values dropped below 1 μg N g^{-1} soil after 28, 29, and 30 months for the Tim Shea, Nunamara, and Boulder sites respectively. As concentrations of NO_3^- in soil solution drop, concentration at the root surface is expected to drop, where a critical level required to maintain fast growth may be reached. For example, 50 μM N was required at the root surface of birch (*Betula verrucosa*) seedlings to maximise growth (Sands and Smethurst 1995). Ammonium is the preferred source of mineral N for *E. nitens*, however, assimilation of NO_3^- has been shown (Garnett 1997, Shedley *et al.* 1993). Hence, NO_3^- concentrations have the potential to indicate N sufficiency.

The Blue Gum, Old Park, Wattle, Wages, Hurds, and Talbot sites that responded to fertiliser had, within unfertilised soils, cold KCl extractable NO_3^- below 1μg N g^{-1} soil and soil solution nitrate below 0.1 mM (except for the Hurds site for which results were lost). The Basalt and Chromeys sites that did not respond had, within unfertilised soils, cold KCl extractable NO_3^- above 1 μg N g $soil^{-1}$ and soil solution NO_3^- above 0.10 mM which may indicate N sufficiency at these sites. Hence for plantations older than three years of age fertiliser should be applied at the beginning of the growing season (i.e. September – October) if values of soil solution NO_3^- are below 0.10 mM and cold KCl extractable NO_3^- are below 1 μg N g $soil^{-1}$. It is possible that N application at the Basalt and Chromeys sites during March to April was too late in the growing season to result in a significant growth response. Applied N may also have leached during the winter, before the next growing season, hence the late fertilisation date is a possible explanation for the lack of significant response at the Basalt and Chromeys sites.

The poor relationship between soil solution and cold KCl extractable NO_3^- (Figure 3.2) casts some doubt on the accuracy of soil solution results. However, both soil analyses were able to separate sites in the same way and it is possible that if the soil solution procedure was repeated, soil solution NH_4^+ and/or NO_3^- may detect more accurately the onset of N deficiency of sites fertilised from planting. The Boulder,

Nunamara, and Tim Shea sites were all responding to N when cold KCl extractable NO_3^- was below 1 μg N g^{-1} soil and soil solution NO_3^- was below 0.1 mM (Figure 6.3).

From results reported in chapter 3, coefficient's of determination (r^2) were high for linear relationships amongst anaerobically mineralisable N, hot KCl extractable N, total, N, total C, and total P. The inclusion of soil analysis results from the September 1997 sampling of a further nine sites maintained these relationships (Table 6.2 and 6.3) for all but total P. These results further suggest that amounts of total N, hot KCl extractable N, and anaerobically mineralisable N reflected the amount of organic matter present at the study sites, but cast some doubt on this relationship with total P.

Application of the budget approach at the field level demands a simple, easily measurable partial budget. With a simpler index of the range of NNM encountered and the use of robust equations for biomass N, a partial budget that measures only NNM supply and uptake demand of N, has the potential for predicting the onset of N deficiency. However more work is required to find such an index and existing equations for biomass N require testing and development with new environments or locations and plantation ages.

Soil analytical methods are simpler and more likely to be adopted as part of standard management practices. Values of total N were used to separate the five sites described in chapter 2, and the Rabbits site, into those that respond before age three years, at age three years and those that had not responded by age three years. Soil solution N and cold KCl extractable N results were used to explain the responses of the remaining eight plantations fertilised after age three year. Hence values of total N in combination with those of cold KCl extractable or soil solution N could retrospectively have been used to guide fertiliser requirements as follows for all of the 14 study sites:

total N 0-0.27%	Fertilise at planting or age one year
total N 0.28-0.51%	Fertilise at age two years
total N >0.51%	Fertilise after age three years, examine soil solution or cold KCl extractable NO_3^- at the beginning of the growing season, (September-October) and apply N fertiliser when NO_3^-

concentrations drop below 1 mM or 1 μg N g^{-1} soil, respectively.

Values of anaerobically mineralisable N may be able to separate responsive and non-responsive sites of three years of age and older if the large temporal variations encountered with this soil analysis in chapter 3 can be explained and predicted.

In summary this study has shown.

- A simpler partial budget with above ground N uptake demand and NNM (0-10 cm) was able to predict fertiliser responses at all of five sites when NNM was estimated *in situ*, but partial budgets predicted fertiliser responses at a maximum of five of 14 sites when NNM was estimated with soil analysis.
- Soil analysis total N had the greatest utility in differentiating age of fertiliser response, being able to separate sites into those that responded before age two years, those that responded at age three years and those that had not responded at age three years. When combined with soil solution or cold KCl extractable NO_3^-, fertiliser responses at all of the 14 study were retrospectively predicted.

6.4 DISCUSSION

For the five sites with measures of *in situ* NNM the simpler partial budget, accounting only for NNM in the top 10 cm and N uptake into the above-ground biomass, accurately predicted fertiliser responses at all five sites. The more complex budget, accounting for surface and subsoil NNM, uptake demand of above-ground biomass, below- ground biomass and losses to litter identified sites that responded by age three years, however two of these sites responded earlier (1-2 years) than this model predicted. When soil analyses were used to predict NNM the ability to predict fertiliser responses reduced. Fertiliser responses were accurately predicted at a maximum of five of the 14 study sites, including only one of the five sites with measures of *in situ* NNM. The ability of soil analyses to divide sites only into those of greater or less than 40 kg N ha^{-1} $year^{-1}$ was too inaccurate to predict responses at 5-10 of the 14 study sites (depending on the soil analysis used). Hence, to construct a usefully predictive partial budget, *in situ* NNM or a soil analysis well correlated to the encountered range of NNM is required.

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The poor relationship between soil solution and cold KCl extractable NO_3^- (Figure 3.2) casts some doubt on the accuracy of soil solution results. However, both soil analyses were able to separate sites in the same way and it is possible that if the soil solution procedure was repeated, soil solution NH_4^+ and/or NO_3^- may detect more accurately the onset of N deficiency of sites fertilised from planting. The Boulder,

Nunamara, and Tim Shea sites were all responding to N when cold KCl extractable NO_3^- was below 1 μg N g^{-1} soil and soil solution NO_3^- was below 0.1 mM (Figure 6.3).

From results reported in chapter 3, coefficient's of determination (r^2) were high for linear relationships amongst anaerobically mineralisable N, hot KCl extractable N, total, N, total C, and total P. The inclusion of soil analysis results from the September 1997 sampling of a further nine sites maintained these relationships (Table 6.2 and 6.3) for all but total P. These results further suggest that amounts of total N, hot KCl extractable N, and anaerobically mineralisable N reflected the amount of organic matter present at the study sites, but cast some doubt on this relationship with total P.

Application of the budget approach at the field level demands a simple, easily measurable partial budget. With a simpler index of the range of NNM encountered and the use of robust equations for biomass N, a partial budget that measures only NNM supply and uptake demand of N, has the potential for predicting the onset of N deficiency. However more work is required to find such an index and existing equations for biomass N require testing and development with new environments or locations and plantation ages.

Soil analytical methods are simpler and more likely to be adopted as part of standard management practices. Values of total N were used to separate the five sites described in chapter 2, and the Rabbits site, into those that respond before age three years, at age three years and those that had not responded by age three years. Soil solution N and cold KCl extractable N results were used to explain the responses of the remaining eight plantations fertilised after age three year. Hence values of total N in combination with those of cold KCl extractable or soil solution N could retrospectively have been used to guide fertiliser requirements as follows for all of the 14 study sites:

total N 0-0.27%	Fertilise at planting or age one year
total N 0.28-0.51%	Fertilise at age two years
total N >0.51%	Fertilise after age three years, examine soil solution or cold KCl extractable NO_3^- at the beginning of the growing season, (September-October) and apply N fertiliser when NO_3^-

concentrations drop below 1 mM or 1 μg N g^{-1} soil, respectively.

Values of anaerobically mineralisable N may be able to separate responsive and non-responsive sites of three years of age and older if the large temporal variations encountered with this soil analysis in chapter 3 can be explained and predicted.

In summary this study has shown.

- A simpler partial budget with above ground N uptake demand and NNM (0-10 cm) was able to predict fertiliser responses at all of five sites when NNM was estimated *in situ*, but partial budgets predicted fertiliser responses at a maximum of five of 14 sites when NNM was estimated with soil analysis.
- Soil analysis total N had the greatest utility in differentiating age of fertiliser response, being able to separate sites into those that responded before age two years, those that responded at age three years and those that had not responded at age three years. When combined with soil solution or cold KCl extractable NO_3^-, fertiliser responses at all of the 14 study were retrospectively predicted.

7. CONCLUSIONS

Soil analysis and budgeting methods were used in an attempt to predict N deficiency of 14 *E. nitens* plantations in Tasmania. As part of the budget approach, *in situ* NNM was estimated at five sites and uptake of N into above- and below-ground biomass was estimated using pre-determined biomass regression equations and estimates of N concentration. N falling in litter was estimated at two moderately productive sites. Results for *in situ* NNM ranged from 13 to 188 kg N ha^{-1} $year^{-1}$. Soil analyses, total N, total P, total C, and hot KCl extractable N results were used to divide five sites into those that had annual *in situ* NNM greater or less than 40 kg N ha^{-1} $year^{-1}$. Estimated uptake into the above- and below-ground biomass ranged from 2 to 67 kg N ha^{-1} $year^{-1}$ in unfertilised sites and 3 to 96 kg N ha^{-1} $year^{-1}$ in fertilised sites. Nitrogen falling in litter ranged from 47 to 56 kg N ha^{-1} $year^{-1}$. The estimated maximum amount of N taken up into the above- and below-ground biomass and lost from the standing biomass was 162 kg N ha^{-1} $year^{-1}$ at the Tim Shea site.

7.1 Budgeting method

Formation of partial budgets where only NNM and N uptake were measured, was able to predict observed growth responses in stem diameter to added N at all of five sites examined when NNM was estimated *in situ*. When soil analysis were used to estimate NNM, observed growth responses in stem diameter were predicted at a maximum of five (36%) of 14 sites. Hence, for a usefully predictive budget NNM may require *in situ* estimation. The labour- and time-intensive measurement of *in situ* NNM, tree measurements and tissue N concentrations need to be reduced if the formation of a partial budget is to be used in broad scale management practises.

7.2 Soil Analysis method

Total N results had greatest utility of total N, total P, total C, anaerobically mineralisable N, hot KCl extractable N, soil solution N, and cold KCl extractable N results for differentiating the age of N deficiency.

Site responses were grouped on the basis of total N determination into those occurring before 3 years (n = 3), those at 3 years (n = 1) and those which were absent within this

time frame (n = 3). Total N levels within these three groups were <0.28%, 0.28-0.51% and >0.51% respectively. Total N values were temporally stable and could therefore be sampled at any time of year. For sites fertilised at a later age, soil solution or cold KCl extractable NO_3^- values accurately separated sites by fertiliser response to N fertiliser at all nine sites examined, where sites having significantly greater stem diameter growth had soil solution NO_3^- concentrations below 1 mM and cold KCl extractable nitrate concentrations below 1 $\mu g\ N\ g^{-1}$ soil. There were large temporal variations in soil solution cold KCl extractable NO_3^-, hence sampling is recommended for the beginning of the growing period. When total N values and soil solution or cold KCl extractable NO_3^- values were used in combination, N fertiliser requirement was accurately (but retrospectively) predicted at all 14 sites. Nitrogen deficiency of sites growing on basalt (n=11), granite (n=1), siltstone (n=1) and alluvium (n=1) were accurately predicted. These soil analysis are simpler than measurements required for the budget approach, and would be suitable for broad scale management practises.

8. FUTURE RESEARCH

8.1 Nitrogen mineralisation

Potential increases in rates of NNM as a result soil disturbance during site preparation and planting may have subsided before measurements began at the study sites. Also, rates of NNM before soil disturbance were unknown. Hence a study where *in situ* measurements commence before and continue immediately after site preparation and planting is suggested. Due to the high variability encountered an increase in the number of replicates is required.

The two to three years of *in situ* measurements provide a valuable data set. These data may be used in the construction or testing of a model that predicts rates of NNM. For example, O'Connell and Rance (1999) model, using anaerobic incubation and weather data may be modified to predict NNM in Tasmania, potentially incorporating an alternative soil analysis without large temporal variations. Where anaerobically mineralisable N is being used to predict NNM, such as incorporation in the model of O'Connell and Rance (1999), preliminary examination of the temporal stability of this index will be required to assess its suitability and evaluate sampling times.

Application of the buried bag technique or a more usefully predictive index of NNM is required to more accurately describe the contribution of the subsoil to NNM.

Little is known of the source or the chemical form of N extracted in various soil analyses. Chemical examination of the various extractants may provide useful information regarding their suitability for use, which may be aided with similar analyses of SOM.

8.2 Partial budget approach

The simpler partial budget utilising NNM (0-10 cm) N supply and above-ground N uptake N demand requires a continued search for a usefully predictive index of NNM to make the partial budget approach a suitable alternative to predict N deficiency in the field. This search may lie in an alternative soil analysis (possible alternatives are

described in Keeney 1982 and Binkley and Hart 1989). Greater success of examined and alternative soil analyses may occur if sites are divided into groups based on soil type, site history and climate. Also, biomass regression and tissue N content regression equations require inclusion of, or testing with data from sites around Tasmania to validate or adapt the equations for general use. The large resource required to measure individual tree diameter and height needs to be reduced if the budget approach is to be applied at the field level, hence a suitable level of sub-sampling of tree dimensions needs to be determined.

Nitrification was undetected in April 1998, at a time when denitrification rates were expected to be high. The procedure involved a single sampling date, hence potential temporal variations in denitrification rates remained unknown. Definitive conclusions regarding rates of denitrification require *in situ* measurements. Hence a procedure such as that of Hilton *et al.* (1994) or Bijay-Singah *et al.* (1989) is recommended for future application. The period when denitrification is most likely to be detected using these methods is March-April, when warmer temperatures occur in conjunction with high soil water content, or during the summer months (November-February) after a large rain event.

Large amounts of N were lost from the top 10 cm, however the significance of losses from the root zone remains unknown. Application of a model such as APSIM (McCrown *et al.* 1996) or LEACHN (Ramos and Carbonell 1991) is required to determine the importance of N losses from the root zone.

8.3 Soil analysis approach

Conclusions for the soil analysis method were limited to observations from 14 sites. Conclusions for sites fertilised within 1 year of planting were limited to six sites and conclusions for sites fertilised at ages 3-10 years limited to nine sites. Due to the limited number of sites examined these conclusions would be strengthened by the inclusion of additional sites. Strengthening the recommendations across climate and fertility gradients across different soil types is required. Hence additional sites representing the range of

encountered plantation ages with representatives from sites allowing evaluation of these variables is required.

This thesis has not dealt with methods of N application (i.e. broacast, spot or band applications) or predicting the amount of additional N required to overcome plantation N deficiency. Research determining the required quantity and best method of N application is required.

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APPENDIX

CHAPTER 2

The following weather data are long-term averages from Bureau of Meteorology (Hobart) and Cooperative Research Center for Temperate Hardwood Forestry (CRC) weather stations close to the study sites. Note, the study sites examined in chapter 2 range in altitude from 390 to 550 meters. Sites 3-6 and a-b encompassing 130-612 m in elevation vary in long-term average monthly maximum or minimum temperatures by not more than 5 °C.

Average Monthly Maximum Temperature (°C)

Site*	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1	24.1	24.3	22.3	18.7	15.8	12.9	12.4	13.7	15.5	18.1	20.1	22.4
2	20.9	21.0	19.9	17.6	15.2	13.3	12.6	13.0	14.2	15.9	17.5	19.4
3	20.4	20.9	19.3	16.5	13.9	11.8	11.1	11.7	13.1	15.1	16.7	18.5
4	21.4	21.6	19.4	15.9	12.5	9.9	9.6	11.1	13.2	15.4	17.4	19.4
5	22.2	22.9	19.1	15.5	12.9	10.6	10.5	11.8	13.4	15.6	16.8	19.7
6	17.6	18.0	15.7	12.4	9.9	7.9	7.2	7.9	9.7	11.9	13.9	16.0
a	20.6	22.2	18.5	14.9	12.5	10.0	9.9	10.0	11.4	15.2	16.4	19.9
b	21.0	21.2	18.1	13.6	11.9	9.1	9.0	10.0	12.3	14.6	15.2	19.2

Average Monthly Minimum Temperature (°C)

Site*	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1	12.1	11.8	10.0	7.3	5.2	2.6	2.0	3.6	5.1	6.9	8.5	10.6
2	12.5	13.1	11.8	9.8	8.3	6.5	5.7	5.9	6.6	7.8	9.4	10.9
3	10.9	11.7	10.7	8.7	7.1	5.2	4.4	4.7	5.4	6.5	8.0	9.4
4	8.2	8.4	7.1	5.6	3.6	1.8	1.2	1.5	2.9	4.1	6.0	7.5
5	8.7	9.3	6.5	5.2	4.2	1.8	2.3	2.3	3.1	4.7	5.5	7.1
6	6.3	6.9	5.9	4.2	2.8	1.4	0.8	0.9	1.7	2.7	3.8	5.2
a	8.6	9.5	6.1	4.1	2.1	1.0	0.9	0.5	0.9	3.1	4.4	6.5
b	7.5	8.2	5.4	3.7	3.1	0.2	0.7	1.7	1.9	4.0	4.2	6.1

Weather Stations, 1-6 = Bureau of Meteorology (Hobart), a-b = Cooperative Research Center

Average Monthly Rainfall (mm)

Site*	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1	47.4	30.6	35.1	53.5	70.1	61.5	80.2	81.2	62.5	52.6	50.6	48.6
2	43.4	49.3	50.5	78.6	98.2	105.3	130.4	110.4	86.8	88.1	71.9	65.8
3	49.1	57.7	57.6	92.8	122.0	133.0	160.1	149.5	117.6	107.0	79.6	76.1
4	67.9	59.7	74.2	98.5	114.8	106.0	122.2	127.9	121.4	121.3	105.0	95.7
5	63.6	82.6	81.0	101.0	104.3	81.8	106.4	136.4	113.4	119.5	114.6	76.3
6	110.1	95.9	123	174.5	215	228.7	250.9	250.7	225.3	203.8	168.6	141.8
7	49.8	79.1	50.1	64.8	164.4	121.5	191.1	157.7	158.8	118.9	100.8	57.0
8	69.2	71.0	74.9	121.0	150.5	165.1	212.7	194.7	146.5	133.4	106.7	102.6
9	81.7	66.6	40.4	146.1	270.1	171.7	336.2	410.9	136.3	194.8	153.3	133.1
10	54.5	58.9	61.8	95.4	125.2	140.2	171.1	156.8	121.6	110.1	84.1	78.1
11	61.8	67.5	73.7	112.7	145.6	158.9	195.1	179.0	142.6	129.4	100.3	91.8
12	81.7	64.5	91.4	123.2	146.6	131.0	148.7	143.1	144.0	138.8	122.7	123.2
13	155.7	116.9	167.5	184.5	248.3	197.5	315.2	267.4	234.9	344.1	206.3	159.7
14	66.6	43.7	53.1	91.9	123.7	109.2	137.8	138.6	99.8	81.1	76.7	94.3
15	45.4	51.3	30.1	58.8	60.1	84.2	41.3	58.8	107.7	63.8	52.8	47.2
16	54.5	58.9	61.8	95.4	125.2	140.2	171.1	156.8	121.6	110.1	84.1	78.1
17	68.5	71.9	76.6	117.4	163.2	169.7	222.8	194.1	151.7	136.3	100.1	91.7
a	137.7	71.3	34.2	74.8	221.4	122.0	116.0	128.3	97.0	83.8	77.9	72.6
b	87.4	91.1	86.8	147.2	124.1	71.2	129.5	155.5	132.5	133.2	163.3	94.3

*Weather Stations 1-17 = Bureau of Meteorology (Hobart)

a-c = Cooperative Research Center

Weather Station (Site) Details

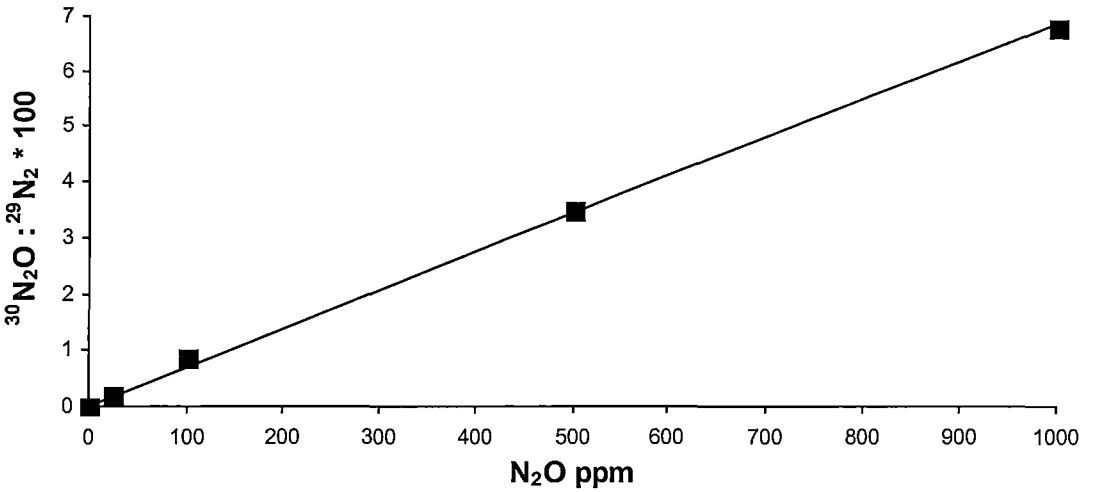
Site	Name	Elevation (m)	Latitude	Longitude	Years Active
1	Launceston	5	41° 25" 15'	147° 07" 22'	1980-present
2	Burnie	8	41° 04" 51'	145° 56" 32'	1944-present
3	Elliott	130	41° 05" 03'	145° 46" 28'	1914-present
4	Maydena	270	42° 45" 49'	146° 35" 50'	1952-1992
5	Maydena	275	42° 45" 30'	146° 37" 21'	1992-present
6	Waratah	612	41° 27" 04'	145° 31" 52'	1882-present
7	Tewkesbury	411	41° 13" 12'	145° 42" 48'	1996-present
8	Tewkesbury	410	41° 13" 53'	145° 42" 22'	1934-1995
9	Parrawe	-	41° 18" 00'	145° 36" 00'	1954-1956
10	Ridgley	277	41° 08" 59'	145° 50" 02'	1909-2000
11	Yolla	343	41° 08" 39'	145° 42" 17'	1905-present
12	Tim Shea	490	42° 42" 18'	146° 28" 09'	1954-1990
13	Mt Field	1230	42° 40" 54'	146° 34" 57'	1987-present
14	Nunamara	425	41° 23" 05'	147° 19" 13'	1948-1994
15	Nunamara	360	41° 23" 51'	147° 17" 57'	1992-present
16	Ridgley	277	41° 08" 59'	145° 50" 02'	1909-2000
17	Hampshire	460	41° 14" 55'	145° 46" 21'	1835-present
a	Nunamara	400	41° 21"	147° 15"	Aug 1993-present
b	Florentine	440	41° 9"	145° 45"	Sept 1993-April 1997

1-17 = Bureau of Meteorology weather stations

a-b = Cooperative Research Center for Sustainable Production Forestry weather stations

CHAPTER 4

Standard curve for estimation of denitrification rates.



CHAPTER 5

Age N fertilised and amount of N applied (maximum rate) at the five sites mentioned in chapter 2.

Site	Age fertilised (years)	Amount of N applied (kg N ha^{-1})			
		Age (years)			
		0	1	2	3
Boulder#	0, 1, 2	25	200	200	0
Basils#	0, 1, 2	25	200	200	0
Nunamara*	1, 2, 3	0	100	200	100
Tim Shea*	1, 2, 3	0	100	200	100
Potters	0	200	0	0	0

Fertilised and unfertilised treatments received 50 kg P ha^{-1} at planting

* Fertilised and unfertilised treatments received 25 kg N ha^{-1} and 43 kg P ha^{-1} at planting

Data used to generate Figure 5.2

	Tissue				
Age	Leaf	Bark	Branch	Wood	Source
1	2.28	1.02	0.90	0.51	Middlesex (Cromer pers. comm)
1	2.16	0.93	0.89	0.40	Nabowla (Cromer pers. comm)
1	1.82	0.99	0.84	0.47	Nunamara (Cromer pers. comm)
1	1.77	0.93	0.99	0.51	Tim Shea (Cromer pers. comm)
2	1.39	0.63	0.52	0.24	Westfield (Cromer pers comm)
2	1.84	0.98	0.93	0.36	Middlesex (Cromer pers. comm)
2	1.02	0.43	0.35	0.13	Nabowla (Cromer pers. comm)
2	1.52	0.78	0.57	0.27	Nunamara (Cromer pers. comm)
2	1.72	0.82	0.62	0.30	Tim Shea (Cromer pers. comm)
3	1.26	0.55	0.40	*	Nunamara (Cromer pers. comm)
3	1.3	0.64	0.55	*	Tim Shea (Cromer pers. comm)
3	1.48	0.51	0.47	0.20	Boulder site sampled September 1996
3	1.68	0.74	0.60	0.26	Basils site sampled September 1996
4	1.2	0.30	0.35	0.10	*E. globulus* (Cromer and Williams 1982)
6	*	0.29	*	0.01	Hurds 1.3 m samples September 1996
6	*	0.27	*	0.06	Blue Gum 1.3 m samples September 1996
6	1.22	0.22	0.16	0.06	*E. globulus* (Bennett *et al.* 1997)
6	1.13	0.23	0.17	0.08	*E. globulus* (Bennett *et al.* 1997)
6	1.10	0.21	0.16	0.07	*E. globulus* (Bennett *et al.* 1997)
6	1.15	0.25	0.35	0.10	*E. globulus* (Cromer and Williams 1982)
7	*	0.35	*	0.07	Talbot 1.3 m samples September 1996
7	1.49	0.19	0.32	0.07	Herbert *et al.* (1991)
8	*	0.30	*	0.02	Wattle 1.3 m samples September 1996
9	1.10	0.35	0.20	0.05	*E. globulus* (Cromer and Williams 1982)
9	*	0.34	*	0.08	Chromeys 1.3 m samples September 1996
10	*	0.31	*	0.07	Basalt 1.3 m samples September 1996

* *Data unavailable*

For Cromer pers.comm, Bennett et al. 1997, and Cromer and Williams 1982 sources, tissue concentrations were determined from average values of sub-samples taken from representative whole trees. Other sources are mentioned in the text.