INVESTIGATION OF THE RELATIONSHIPS BETWEEN BIOMASS REDUCTION, SOIL DISTURBANCE, SOIL NUTRIENTS AND WEED INVASION IN BASALT PLAINS NATIVE GRASSLAND REMNANTS IN VICTORIA, AUSTRALIA

WIPULAL SARDHA WIJESURIYA
(B. SC., M. SC.)

DOCTOR OF PHILOSOPHY

1999

INVESTIGATION OF THE RELATIONSHIPS BETWEEN BIOMASS REDUCTION, SOIL DISTURBANCE, SOIL NUTRIENTS AND WEED INVASION IN BASALT PLAINS NATIVE GRASSLAND REMNANTS IN VICTORIA, AUSTRALIA.

Thesis submitted in fulfilment of the requirements
for the degree of Doctor of Philosophy
of Victoria University

by

Wipulal Sardha Wijesuriya (B.Sc., M.Sc.)

School of Life Sciences and Technology
Faculty of Engineering and Science
Victoria University
December 1999.

This thesis is dedicated to my mother

and

my father (passed away in 1974).

Derrimut grassland reserve in Victoria, Australia.

Declaration

The research in this thesis has not been previously submitted for a degree or diploma in any University. The thesis contains no material that has been previously published or written by another person, except where due reference is made in the thesis itself.

[redacted]

Wipulal Sardha Wijesuriya

Acknowledgments

It is with great pleasure that I express my sincere thanks and gratitude to all who helped and supported me to complete this work.

I record my most sincere appreciation and gratitude to my supervisor Dr. Colin Hocking, for his continual encouragement and guidance in conducting this research and in preparation of the thesis.

My special thanks go to Prof. Bob Fairclough, Head, School of Life Sciences and Technology for providing me travel funds to attend conferences and also giving me opportunity to teach at the School of Life Sciences and Technology. This was invaluable.

My appreciation also extends to Ass. Prof. John Orbell, former Head, Department of Environmental Management, for providing me departmental scholarship when I began postgraduate studies at VUT.

Special thanks go to Dr. Russ Swan, School of Life Sciences and Technology, for his valuable comments on conference proceedings and research publications.

This study was jointly funded by grants from Department of Natural Resources and Environment (DNRE) and BHP (Methanol Research branch), Laverton, Victoria.

I am grateful to Bram Mason, Research Officer, grassland ecology research unit, VUT, for his valuable support in setting up experimental plots and laboratory work.

Thanks must also go to Ben, Joanne, Matt, Megan, Meredith, Paul, Rachael and Ruben, grassland ecology research group, VUT, for their valuable voluntary support for field work and laboratory work.

My appreciation also extends to all present and past technical staff at School of Life Sciences and Technology, for their enthusiastic support and co-operation.

My warmest appreciation goes to my wife Sriyani. Her excellent support and encouragement throughout were invaluable, as was her care for our little daughters all day and helping me with field work, laboratory work and thesis preparation.

I extend my sincere gratitude to my mother who has always blessed me and my family. My special thanks also extend to my brothers and sisters. They always encouraged me to complete this work.

I am also grateful to my wife's extended family who looked after her and our daughters during my absence.

Finally, I thank my little daughters Chathuri and Yasaswi, for not being naughty to mum.

Abstract

The investigations reported in this thesis examined the relationships between biomass reduction (burning and mowing), soil disturbance, soil nutrient levels (N, P and K) and weed invasion in native grassland remnants in Victoria, Australia.

The study was carried out at three native grassland remnant sites, Derrimut grassland reserve, Laverton grassland reserve and Victoria University of Technology, St. Albans campus grassland reserve on the western edge of Melbourne, Australia. These grassland remnants belong to the Western Basalt Plains grassland community which is listed as threatened in Victoria, and which forms part of the lowland temperate grasslands which once extended across south eastern Australia. A major threat to the survival of these communities is invasion by a variety of weeds. Weed invasion is often markedly enhanced by disturbance of the soil due to ploughing and overgrazing. It is known that some type of biomass reduction (burning, mowing or grazing) is required to prevent the dominant native perennial tussock grasses from over shadowing the diversity of forbs that grow between the tussocks, but the effects of various biomass reduction methods on weediness, and the underlying causes of weediness, have not been studied in any depth.

The levels of extractable and total nutrients (N, P and K), pH and organic carbon content at 2 - 5 cm soil depth were estimated at each remnant grassland site. Both total and extractable nutrient levels were found to be low in native grasslands compared to forest ecosystems. For example, extractable nitrogen (N) was about 12 times lower, phosphorus (P) about four times lower and potassium (K) about two times lower in lowland native grasslands than in rainforest ecosystems. However, the levels of mineralizable nitrogen (N) and rates of mineralization of nitrogen (N) in grasslands were not greatly different to forest ecosystems. These results strongly suggest that there are substantial amounts of potentially available nitrogen locked up in the vegetation of the native grasslands.

Physical soil disturbance in the native grassland remnants was found to lead to rapid and massive increases in extractable nutrients (N, P and K). This was most likely due to rapid mineralization of the plant materials killed by digging. Mineralization was likely to have been promoted by changed micro-climatic conditions following digging, including increased aeration, temperature, moisture and light in disturbed soil plots. The rate of *in-situ* mineralization of nitrogen was more than two times (> 2) greater in soil disturbed (dug) plots than in undisturbed (undug) plots, and after 70 days there was 20 times more extractable nitrogen (NO_3^- - N + NH_4^+ - N) and about two times more extractable phosphorus (PO_4^{3-} - P) in disturbed (dug) plots than in undisturbed (undug) plots.

High levels growth weeds were observed in disturbed native grasslands plots compared with undisturbed areas, a result consistent with numerous previous studies. The addition of extra nutrients (N and P) to disturbed plots further promoted weed growth, suggesting that the high growth of weeds on disturbed sites was due in part to high level of available nutrients in the soil. Depleting nutrients by adding a carbon source (sugar) decreased weed growth in disturbed soil. The size (i.e. diameter) of the soil disturbance affected final levels of extractable nutrient. Higher extractable nitrogen (N) and phosphorus (P) levels were found in larger disturbance plots than in smaller disturbance plots. Larger disturbances also produced higher densities of weeds than smaller disturbances, most likely because larger disturbances contained higher levels of available nutrients.

Addition of nutrients (N + P) to native grassland plots with undisturbed soil, but with high densities of annual weeds (grasses and flatweeds) also promoted growth, leading to increased size of individual plants of these weeds compared with untreated plots. However, the actual densities of weeds did not increase as a result of nutrient addition. These results suggest that the levels of available nutrients in undisturbed grasslands can influence the levels of weediness, at least in terms of total biomass production per plant..

The effects of grassland management practices (burning and mowing) on extractable nutrients (N, P and K) in the soil in different seasons, and on levels of weed growth were also investigated. Levels of extractable nutrients were markedly variable across seasons. Different seasonal patterns of extractable nutrients were also observed between burning and mowing treatments. In addition, the seasonal patterns were different for different nutrients. This may have been due to the drought conditions persisting over two years of the study, leading to partial plant death and mineralization. The extractable levels of all nutrients (N, P and K) increased progressively over three years (from 1996 to 1998) in all seasons measured (autumn spring and summer), for most treatments. The extractable nitrogen (N) in burnt plots was significantly higher than in control (unburnt) and mown plots after three years in all seasons except spring. Similar patterns were observed at both sites. The extractable phosphorus (P) was significantly lower in annually burnt plots than in control and mown plots in autumn and spring after three years but similar levels of P were found in summer across all treatments. The extractable levels of K in burnt plots was higher in spring (soon after burning in the previous autumn) but became lower in summer and autumn (in the following year) probably as a result of losses by leaching.

The burnt once treatment resulted in lower extractable potassium (K) and phosphorus (P) compared with control treatments, but extractable nitrogen (N) was not affected. Mown once treatments had no significant effect on any of extractable levels of the nutrients N, P and K.

The outcomes of the investigation are currently being applied to the management and rehabilitation of lowland grassland remnants. Most previous studies of lowland grassland rehabilitation and management have assumed that the key determinant of competition between native and exotic plant species in lowland grasslands is above-ground cover. The results reported in this thesis clearly show that below-ground competition for nutrients is also important in determining the extent of exotic and native plant growth in intact and disturbed areas. The successful establishment of native species during revegetation, with low levels of weed growth, will depend on the extent to which below ground growth of natives is achieved to lower available nutrient levels quickly, and prevent large scale growth of exotics. The management of lowland grassland remnants with low levels of flatweeds and annual grasses will likewise depend on the maintenance of a healthy sward of native vegetation which can keep available nutrients in the soil at low levels, especially when above-ground cover is removed by biomass reduction actions such as burning, mowing and grazing.

List of Tables

List of Figures

Table of contents

Chapter 1 Significance and management of lowland native grasslands

1.1 Status of Australian grasslands

According to McDougall and Kirkpatrick (1994) temperate lowland native grasslands are the most threatened ecosystems in Australia. While some of Australian's native grasslands in less settled areas are relatively intact (eg. subtropical savanna grasslands of Nothern Australia (Kirkpatrick *et al.* 1995), most grassland ecosystems in regions of high agricultural activity have been grossly fragmented (Hobbs 1987). The destruction of native grasslands is not unique to Australia. There has been a dramatic decline of native grasslands across the world due to their suitability for, and sensitivity to, agriculture and the rapid growth of industrialisation and urbanisation. Approximately 20% of the world's natural vegetation cover was once made up of some type of grassland (Lamp *et al.*1990). The pampas of Argentina, the prairies of America and the chalk grasslands of Europe have all been drastically depleted by human activities, for example, Holland has lost 30% of its plant species during the last century (Green 1990). In Britain ten vascular plants and four invertebrates have become extinct during the same period and another 149 plant and 14 invertebrate species are endangered (Green 1990).

Grassland is a vegetation type dominated by grasses but containing many broad leaf herbs (forbs). According Moore's (1964) definition, natural grassland is a plant community in which the dominant species are perennial native grasses, there are few or no shrubs, and trees are absent. In a wider sense, any plant community, whether natural or developed by humans, in which grasses provide a substantial proportion of the feed for domestic stock can be called a grassland (Groves & Williams 1981). Grasses belong to the family Poaceae (Graminaea) which is estimated to consists of 620 to 650 genera containing about 10,000 species (Lamp *et al.*1990). In addition to role of ecological importance of grasslands, agriculturally they are arguably the most important plant communities for European - style settlement.

The native temperate lowland grassland of south - eastern Australia, across New South Wales, The Australian Capital Territory, Victoria, South Australia and Tasmania, are amongst the most fragmented and degraded ecosystems in the world. In each of the eastern Australian states in which they occur lowland temperate grasslands are recognised in appropriate environmental legislation as threatened with extinction. Draft proposals for listing of these communities as threatened under the federal government Endangered Species Protection (ESP) Act (1995) are also in preparation.

1.2 Types of Grasslands in Australia

Groves and Williams (1981) propose four major types of grasslands for Australia.

1. **Arid tussock grassland** - Dominated by Mitchell grasses (*Astrebla* spp.), distributed in Queensland, Nothern Territory and northern western Australia, receiving 200 - 500 mm annual rain fall.

2. **Arid hummock grasslands**. Dominated by *Triodia* and *Pletrachne* in areas receive less than 200 mm average annual summer and/or winter rain fall. They have a vary extensive distribution throughout arid Australia.

3. **Coastal grasslands**. Dominated by *Sporobolus* and *Xerochloa* and confined to the tropical summer rainfall region.

4. **Sub-humid grasslands**. These are sub devided into three groups:

a. Tropical. Grasslands dominated by *Dichanthium* and *Eulalia* and sometimes by *Bothriochloa* and *Heteropogon*, in eastern and northern Queensland in regions with predominantly summer rain.

b. Temperate. Grasslands with an irregular distribution from north of Adelaide around the zone of 500 to 100 mm average annual winter and /or summer rainfall of southeastern Australia to northern New South Wales. Dominant genera are *Themeda, Poa* and *Stipa*. Temperate grasslands are also known as lowland native grasslands.

c. Sub alpine. Confined to the cold and wet mountain regions of the Monaro region of Southern New South Wales, northeastern Victoria and the central plateau of Tasmania. Dominant genera are *Poa* and *Danthonia*.

1.2.1 Temperate Lowland native grasslands in Victoria

At the time of the first European settlement in Australia, lowland areas of south-eastern Australia had one of the largest areas of native temperate grassland in the world covering about two million hectares (Williams *et al.*1995). McDougall and Kirkpatrick (1994) suggest that there are now only about 10, 000 hectares of lowland native grasslands remaining in south-east Australia, representing about 0.5 % of the original area.

Five main biogeographic regions of open lowland grassland communities in Victoria are recognised (McDougall & Kirkpatrick 1994; Williams *et al.* 1995). These are: Western Basalt Plains communities, Gippsland community, Omeo Plains Community, Riverina Communities and Wimmera Communities. Western Basalt Plains covers the major area of lowland native grassland community. The Basalt Plains grasslands which once stretched from the northern and western areas of Melbourne to almost the south Australian border, now constitute perhaps the most threatened of these lowland grassland communities with less than 0.5% remaining (Lunt 1991). These remnants are located in small, isolated pockets along the roadside and railway lines, in cemeteries and in uncultivated lightly grazed paddocks (Groves 1965; Lunt 1991; Craigie & Stuwe 1992).

Victoria's once extensive natural grasslands were dominated by perennial, tussock forming grasses such as *Themeda, Stipa, Danthonia* and *Poa* (Lunt 1991). It is likely that *Themeda* dominated grassland communities contained few native annuals (Willies 1964), although the annuals were probably more prevalent in the less productive northern and Wimmera plains grasslands (McDougall and Kirkpatrick 1994). The study reported in this thesis was undertaken in two lowland (temperate) grassland remnants in Victoria on the western edge of Melbourne. The minimum annual temperature ranges between 0°C and 5°C and the maximum between 31°C and 35°C. The annual rainfall varies between 294.6 mm and 809.4 mm (Bureau of Meteorology, Melbourne).

1.3 Grassland management practices

Burning and grazing are the principle methods used to manage temperate lowland grasslands. Mowing is also sometimes carried out on a small scale. Each of these methods removes above ground biomass and creates small open spaces between tussocks which encourage the growth of soil micro-organisms and many native plant forb species which constitute the biodiversity found in grasslands (Kirkpatrick *et al.* 1995). The individual effects of burning and grazing management practices are discussed in detail below. The effects of burning and mowing on soil and vegetation parameters are discussed in more detail in chapter eight.

1.3.1 Burning

Burning of grasslands, or other ecosystem, directly and indirectly affects soil temperature, organic matter, soil nutrients, soil pH, soil micro climate (soil - atmosphere interface), soil micro-organisms and above ground fauna and flora (Daubenmire 1968).

Grasslands have traditionally been burnt for ecological and agricultural purposes. Agriculturally, there are a range of reasons for burning grasslands (Moore 1970) including: (1) to remove accumulated inedible grass; (2) to stimulate the growth of succulent and nutritious new grass; (3) to influence the distribution of animals on the range; (4) to remove fire hazards; and (5) to prepare a seed bed for natural or artificial seeding of desired forage species.

Fire is an important ecological factor in many of the world's grasslands, particularly in semi-arid regions where burning, annually or every few years, is a well established practice (Campbell 1960). Ecologically, fire helps to promote plant species diversity in most grasslands. Stuwe and Parsons (1977) and Robertson (1985) have concluded that regular burning of lowland Australian grasslands dominated by *Themeda triandra* (Kangaroo Grass) maintains the diversity of plants by preventing the Kangaroo Grass from out - competing other native plants. Lemon (1949) and

Ramsay and Innes (1963) have pointed out that fire in a natural community may enrich flora when none of the species already on the habitat are destroyed but the stand is opened up to allow opportunistic species to invade. The floristic enrichment in the grasslands after fire is due to removal of heavy litter which accumulates in long unburnt grasslands, preventing seedling establishment of many inter-tussock species (Heady 1956; Weaver & Rowland 1952).

Fire changes the physiological activity of some plant organs. Mes (1958) found that fire increases activity in the roots of many burnt plants. This in turn often stimulates the uptake of nutrients. The flowering activity of some grass and forb species increased (eg. *Themeda triandra* in Africa) and some decreased (*Poa colensoi* in New Zealand) in response to fire (Sewell 1948). O'Connor and Powell (1963) found that burning can shorten average leaf length of grass species but at the same time can increase the number of tillers. Many grassland species also re-sprout vigorously after fire eg. *Themeda triandra*, daisy species such as *Calocephalus citreus* and lilies such as *Arthropodium* species.

Daubenmire (1968) pointed out that species growing together may respond very differently to the same fire. Some species may be in an active state of growth and specially susceptible at the time of burning, where as others may be dormant and less susceptible. The survival of seeds and plants after fire depends on several factors. *Celmisia spectabilis* from New Zealand tussock grassland possesses a fire resistant strategy in the form of moisture holding sheaths made up of living and dead leaves (Barker 1953). The position of seeds and buds at the time of burning often determines the extent of damage (Daubenmire 1968), and a general pattern which has been observed is that buds or seed pods in higher position are more susceptible to damage. Bently and Fenner (1958) also pointed out that the seeds in the upper part of leaf litter or still in the florescences are damaged by fire, but seeds located at or below ground level are more likely to survive.

In summary, when a grassland is burnt, the season, intensity, frequency and pattern of the fire as well as the biology and history of individual species are all important factors which influence the survival, recruitment and regeneration of native grassland and exotic plant species. More specific information about the effects of burning on native grassland ecology is discussed in later appropriate chapters.

1.3.2 Mowing and Grazing

Mowing

Mowing is another method for reducing the build up of dead biomass in the perennial grasses of native grasslands. While mowing is a relatively modern anthropogenically constructed influence on grasslands, its importance as a management technique should not be overlooked, especially where fire or grazing is not possible, and biodiversity needs to be maintained. As a general rule, mowing is considered to be less favourable to lowland native grasslands than burning (Chan 1990, Kirkpatrick *et al.* 1988). However Scott (1951) in South African grasslands and West (1965) in subtropical and tropical African grasslands showed that annual mowing maintained grassland in much the same condition as annual burning. Scott (1951) however, points out that while mowing may be superior to fire in most respects, fire is necessary in alternate years to prevent the invasion of woody plants in South African grasslands. Overall, very little research in recent times has been conducted on the effects of mowing on temperate grasslands.

Grazing

Grazing is another way of managing biomass build up in native grasslands which may often result in effects which are more natural than mowing. Morcom (1990) found that in lowland native grasslands in Victoria there was a progressive reduction in the diversity of native plant species and an increase in cover and percentage of exotic plants, with increasing grazing intensity. He found that this was because of degradation of ground vegetation, competition for resources, trampling and destruction of the litter layer and soil compaction. Morgan (1994) suggests that native herbivores would have had an important effect on the ecology of South Eastern Australian lowland native grasslands. This would have been due to selective grazing by herbivores. Eastern grey kangaroo have been shown to be highly selective grazers at Gellibrand Hill Park, Victoria (Robertson 1985). Morgan (1994) has pointed out that where grazing of the dominant grass occurs, subordinate species such as forbs may benefit due to relief from above ground competition for light. Some types of grazing have been observed to lead to an increase in unpalatable exotic species in Australian lowland grasslands (Morgan 1994). Robertson (1985) found that *Arctotheca calendula* (a rosette without prickles) and *Trifolium* spp. (clover) became abundant in an Australian temperate grassy woodland because the kangaroos that grazed the community favoured eating grasses over dicot species.

Comparison of Mowing and Grazing

Wendin and Tilman (1992) have pointed out that, while mowing and the removal of hay is not as natural a process for biomass reduction as grazing, some type of mowing regime is often more effective at maintaining native species. Mowing and removal of hay reduce above ground biomass and subsequently reduce litter and nutrient flow to the soil. The opening of the grassy sward through removal of hay in a temperate lowland grassland in the Australian Capital Territory has also been found to allow better growth potential of native grasses and to promote tussocking (Sharp 1996).

1.3.3 Seasonal and Other Effects of Burning, Mowing and Grazing

The effects of burning, mowing and grazing on native grasslands appear to depend on the season of the year. The effects can be reversed at different seasons (Daubenmire 1968). O'Connor and Powell (1963) found that responses to burning and to mowing were same in *Chionochloa rigida* grassland in New Zealand. For a more detailed discussion of seasonal effects of burning, mowing and grazing on native grasslands see chapter eight.

1.4 Threats to south-eastern lowland native grasslands

Most of the remaining remnants of south-eastern lowland native grasslands in Australia have been affected by heavy grazing, inappropriate frequency and season of burning, soil disturbance, changing soil nutrient levels (eg. application of fertilisers, herbicides and other chemicals), and the introduction of exotic pasture species and other weeds (McDougall & Kirkpatrick 1994, Williams *et al.* 1995). The threatening effects of these factors are discussed below.

1.4.1 Changes to Burning and Grazing Regimes

A number of endangered plant species in Australian lowland grassland remnants depend on specific burning regimes for regeneration (Scarlet & Parsons 1982). Sucling and MacFarlane (1984) propose that grasslands require less frequent fires than the regimes imposed by prescribed, hazard - reduction burning. Burning can also promote many exotic species such as annual grasses and thistles, particularly in degraded remnants (Lunt 1990 c,d). The reasons for this have been unclear until recently. The discovery that *Themeda triandra* undergoes a process of senescence after seven to eight years in some Australian grassland remnants has shed light on this response to burning (Lunt & Morgan 1998). Burning senescent *Themeda triandra* causes significant plant death, especially if carried out in dry autumn conditions, leading to opportunities for invasion by exotic weeds (Henderson & Hocking 1998).

Over-grazing also promotes weed invasion in grassland ecosystems. According to Craigie and Stuwe (1992) constant and intensive grazing reduces the number of native plants species and encourages exotic species. They also pointed out that the trampling associated with stock grazing can cause significant damage to vegetation and soil structure.

Intensive grazing of native grasslands causes degradation of the litter layer, soil compaction and soil erosion especially under dry conditions (Dept. of Conservation and Environment, 1992). Groves and Williams (1981) found that if grazing occurred soon after a fire and continued then the regenerative capacities of some grasses were severely depleted.

1.4.2 Weeds

The invasion of native grasslands by introduced weeds is a widespread problem in Australia and through out the world. Lowland native grassland remnants in Victoria appear to have a high level of resistance to invasion when appropriately managed (Lunt & Morgan 1998). However, native grassland vegetation is easily invaded by exotic plants when it is exposed to soil disturbances, intensive grazing or increased nutrients (Craigie & Stuwe 1992).

Weeds are not always easy to define. Some plant species are readily recognised as weeds because of their general undesirableness, others are not as readily identifiable as weeds because their level of desirability depends on how they affect the interest of an individual or community. Certain plants my be described as weeds at one time or in some situations but as useful plants at another time or in other situations. For example, Safron thistle (*Carthamus lanatus*) is classified as a weed but its seed provides useful feed for sheep in late summer and in dry autumn (Michael 1970). Ecologically weeds can be classified as non-indigenous invaders of naturally occuring communities (Heywood 1989). The recent Victorian State Government enquiry into pest plants (Victorian State Government 1997) defines weeds as plants which are unwanted in a number of given situations. Pest plants are further divided into two groups - environmental weeds and agricultural weeds. The definition for environmental weeds is " plants which invade native vegetation, usually adversely affecting regeneration and survival of the indigenous flora and fauna. There are many types of environmental weeds. These include trees, shrubs, vines and creepers, grasses, bulbs, tubers and rhizomes, herbs and succulents and aquatic plants". Agricultural weeds are those plant species which "threaten crop, horticultural and pasture production".

Some of the reasons why individual plant species are successful ecological weeds when competing with native plants include: their high disease resistance (few natural predators), their low palatable to herbivores, their competitiveness for light, water, carbon dioxide gas, nutrients and growing space, their rapid growth rate, their allelopathic effect on competing species, their large production of seeds, their effective long distance seed dispersal mechanisms, the longetivity of their seeds in the soil seed bank (eg. some *Amaranthaceae* sp. of weeds are viable for 40 years), their ability to adapt to a wide range of environmental conditions and to tolerate adverse conditions, their successful vegetative reproduction and their broad cytological and genetical variability (Smith & Taylor 1986).

Many of the worst weeds are plant species with C_4 photosynthetic mechanisms sometimes known as summer growing species. These species have a specialised leaf anatomy and an extra stage in their photosynthetic process. This combination allows C_4 species to take up carbon dioxide at lower levels (ie. stomata more closed) and speeds up the production of sugars, and makes the plant grow faster than ordinary C_3 plants under conditions of temperature and water stress. The plant families containing most weed species are Asteraceae (Compositae), Poaceae, Fabaceae, Solanaceae, Cactaceae, Brassicaceae, Onagraceae and Rosaceae (Haywood 1989). The Asteraceae are regarded as one of the most advanced families containing high number of weed species, many of which are spread throughout temperate regions of the world (Haywood *et al.* 1977). Their success derives largely from the development of biological features which both ensure survival under adverse conditions and allow high reproductive rates. Chemical factors are also important in their success in providing protection from overgrazing. For example, common groundsel (*Senecio vulgaris*) leaf tissues contain toxic pyrrolizidine alkaloids (Haywood *et al.* 1977). Next to the Asteraceae family, the Poaceae (grass family) contain many weeds spread through out the world. The highly evolved inflorescence containing reduced and aggregated flowers, coupled with a series of dispersal mechanism in the flowers, and associated parts, together with a diversity of habitat has been largely responsible for their successful role as weeds.

The weed species found in my experimental plots were mainly annual grasses (eg. *Briza maxima*, *Briza minor*, *Vulpia bromoides*, *Aira caryophylla*, *Bromus cartharticus*), perennial grasses (eg. *Romulea rosea*), annual herbs (eg. *Trifolium campestre*, *Hypochoeris glabra*, *Picris echioides*, *Sonchus oleraceus*) and biannual herbs (eg. *Cirsium vulgare*). Out of these, *Sonchus oleraceus* and *Cirsium vulgare* belong to the thistle group. Most of the weeds encountered in field experiments belong to the two most common families from which weed species derive: Asteraceae and Poaceae. Very few investigations have been undertaken of how the biology of these species allows them to be successful invaders of Australian lowland grassland remnants. For a more detailed discussion of what influences weed growth in Australian lowland grassland remnants, see chapters five and seven.

1.4.3 Soil nutrients

Soil nutrient levels are major factors in determining species composition and diversity of communities of a site (Whittaker 1965; Tilman 1982; Parrish & Bazzar 1985).

In grassland ecosystems the nutrient status of the soil has been found to be a major factor which limits the growth of plants (Groves & Williams 1981). Many of the exotic species that have invaded native *Themeda* and *Danthonia* dominated grasslands show a greater response to the addition of nutrients than the native species (Groves *et al.* 1973; Fisher 1974). Extensive anecdotal evidences show that most Australian native plants are adapted to low nutrient levels. Therefore, the application of inorganic fertilisers such as superphosphate and nitrogenous fertilisers, herbicides and other chemicals increase soil nutrient levels and assist weeds to dominate over native plants (Lunt 1991; Craigie & Stuwe 1992).

There are sixteen elements identified as essential for plant growth (Solomon *et al.* 1996). Nine of these are required in fairly large quantities and are known as macronutrients. These include carbon (C), hydrogen (H), oxygen (O), nitrogen (N), phosphorus (P), potassium (K), sulfur (S), calcium (Ca), magnesium (Mg). The remaining seven elements are known as micronutrients, and which are needed in trace amounts. These include iron (Fe), boron (B), manganese (Mn), copper (Cu), molybdenum (Mo), chlorine (Cl), and zinc (Zn).

Out of nine macronutrients, only three elements are most often limiting for plant growth. They are nitrogen (N), phosphorus (P) and potassium (K). These were the three elements which were the focus of the study outlined in this thesis. There are two main ways that these can be conceptualised in the forms they take in soils under natural ecosystems. These are “available” form and “total” form.

1.4.3.1 Available nutrient levels

Available nutrients are essential elements in the root zone that are in a chemical form that can be readily absorbed by plants (Keeny & Nelson 1982). These includes ionic or inorganic form of nutrients (eg. NH_4^+, NO_3^-, NO_2^-, $H_2PO_4^-$, HPO_4^{2-}, PO_4^{3-}, K^+, Ca^{2+}, Mg^{2+} etc.). Levels of available nutrients are strongly correlated with nutrient uptake and limitations of plant growth (Vitousek 1985). The levels of these ions which are available in the soil at a given time are influenced by several soil parameters, including : soil moisture, temperature, aeration, pH and organic matter content. Each of these factors also directly affects the population size and activity of soil microorganisms, which in turn can alter the available nutrient levels in the soil. Apart from microbial activity, the levels of available nutrients in the soil are strongly influenced by the mineral composition of parent rock material, its rate of breakdown and the texture of the resultant soil (Black 1968, Brady 1984). The effects of soil parameters on the availability of individual nutrients (N, P & K) will be discussed in detail later in section 1.4.3.

1.4.3.2 Total nutrient levels

The total amount of each nutrient N, P and K in soil provides a measure of the quantity of substrate undergoing decomposition. This includes the sum of available and unavailable forms of each nutrient (unavailable = forms not directly available by plants). Unavailable (mostly insoluble or bound) forms of nutrients can be either organic compounds or inorganic compounds. Unavailable forms of nitrogen (N) mainly occur as organic compounds (eg. proteins and nucleic acids). Non-exchangeable ammonium (NH_4^+) is an example for inorganic form of N. Non-exchangeable NH_4^+ accounts for 4% - 8% of total nitrogen in most ecosystems (Black 1968). Unavailable phosphorus (P) consists of inorganic and organic forms. Inorganic forms of phosphorus in the soil occur as either insoluble or poorly soluble forms. Most inorganic forms of phosphorus are unavailable to the plants (Paul & Clark 1989).

1.4.3.3 Macronutrients essential for plant growth

Nitrogen (N)

Nitrogen is usually the most limiting nutrient for the plant growth. Nitrogen occurs in different forms in the soil, including : the gases nitrogen (N_2), nitrous oxide (N_2O) and nitric oxide (NO) and the ions ammonium (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-). The gaseous forms of nitrogen are present only in traces (Paul & Clerk 1989; Black 1968). The ionic or available forms (NH_4^+, NO_2^- & NO_3^-) are found in soil solutions. The available forms of nitrogen usually constitute less than 2% of total nitrogen (Black 1968). NO_2^- and NO_3^- are soluble and occur as freely diffusible ions in the soil solution. There are three forms of NH_4^+ in the soil. They are NH_4^+ in the solution, exchangeable NH_4^+ and non exchangeable NH_4^+. Most of NH_4^+ occurs in exchangeable and nonexchangeable forms. Only a little occurs in the soil solution. These three forms are in an equilibrium as follows (Black 1968 & Brady 1984).

$$\underset{\text{(solution)}}{NH_4^+} \Longleftrightarrow \underset{\text{(exchangeable)}}{NH_4^+} \Longleftrightarrow \underset{\text{(non exchangeable)}}{NH_4^+}$$

The rest of nitrogen in the soil occurs in organic form. Three major organic forms of nitrogen are present in the soil. They are proteins (live and dead plant and animal tissues), microbial cell wall constituents, such as chitin and peptidoglycans and the nucleic acids. During the process of mineralization these organic forms of nitrogen are converted to the ammonium ion (NH_4^+) by soil microorganisms. Certain soil microorganisms can convert atmospheric nitrogen gas (N_2) into soluble ammonium ion form. This process is known as nitrogen fixation. The associated organisms include bacteria (e.g. *Rhizobium* sp., *Azotobactor* sp., *Clostridium* sp.), blue green algae (e.g. *Notoc* sp. & *Anabaena* sp.) and Actinomycetes (e.g. *Frankia* sp.) (Brady 1984). The enzymatic oxidation process known as nitrification converts NH_4^+ to NO_3^- by certain soil organisms. Oxidation takes place in two coordinated steps. In the first step, NH_4^+ is converted to nitrite (NO_2^-) by specialised soil bone bacteria *(e.g. Nitrosomonas europaea, Nitrosolobus multiformis, Nitrosospira briensis*). In the second step NO_2^- is converted to nitrate (NO_3^-) by other soil bone bacteria such as *Nitrobacter winogradskyi* and *Nitrospira gracilis* or similar species (Sprent 1987).

Phosphorus (P)

Phosphorus is the second most limiting nutrient for plant growth next to nitrogen (Miller & Donahue 1995). Phosphorus in soils occurs principally as orthophosphates. These phosphates can be divided into two groups, inorganic and organic forms. Inorganic form of phosphorus are orthophosphates ($H_2PO_4^-$, HPO_4^{2-} and PO_4^{3-}) of iron (Fe), aluminium (Al) and calcium (Ca). Most inorganic soil phosphates are insoluble or very poorly soluble. When phosphorus is added to soil as soluble salts, it is often incorporated or "fixed" into insoluble compounds (Paul & Clark 1989). There are three forms of organic phosphorus in the soil. They are phytin and phytin derivatives, nucleic acids and phospholipids. Phytin is the most abundant organic form of phosphorus in the soil. It generally makes up 30% - 40% of organic phosphorus (Brady 1984). The relative proportion of the phosphorus in the organic and inorganic categories vary widely between soil types. The organic phosphorus tends to increase and decrease with the content of organic matter and hence is comparatively low in subsoils and high in surface soils. Organically bound phosphorus usually constitutes 30% - 50% of the total phosphorus in most soils, although it may range from 5% to as high as 95% (Paul & Clark 1989; Black 1968).

Plants meet their phosphorus requirement for growth out of the solution pool of phosphorus. Solution phosphorus equilibrates with labile phosphorus (exchangeable forms of phosphorus). Soil micro-organisms contribute to the renewal of the solution phosphorus pool both by acting to solubilize bound forms of phosphorus, and by mineralization (decomposition) of organic phosphorus.

Potassium (K)

Potassium (K) is the most abundant metallic element (2% - 3% dry weight) in plant cells (Black 1968; Miller & Danahue 1995). Potassium plays many essential roles in plants metabolism. It is an activator of over sixty enzymes responsible for plant processes (Miller & Danahue 1995), such as starch synthesis, nitrate reduction and sugar degradation. Its relatively high concentration in plant cells helps to regulate the opening and closing of stomata in leaves and the uptake of water by root cells.

Most of the potassium in soils is present in minerals classified as feldspars and micas. Apart from this, the decomposition of dead plant materials supplies significant amounts of soil potassium.

Three major forms of potassium in the soil. They are nonexchangeable, exchangeable and soil solution (soluble) potassium. Exchangeable and soil solution potassium mostly occur as the ion K^+. This is the form of potassium taken up by plants. Non exchangeable potassium makes up about 99.6% of total potassium in the soil (Black 1968). Minerals containing potassium are quite resistant to weathering and supply relatively insignificant quantities for plant growth. The remaining 0.4% includes both exchangeable and water soluble forms of K^+. The water soluble K^+ usually constitutes only a small part of the total of water soluble and exchangeable forms. The three forms of potassium in the soil are in equilibrium as follows.

$$\begin{array}{c}\text{nonexchangeable}\\\text{potassium}\end{array} \Longleftrightarrow \begin{array}{c}\text{exchangeable}\\\text{potassium}\end{array} \Longleftrightarrow \begin{array}{c}\text{solution}\\\text{potassium}\end{array}$$

1.4.3.4 Mineralization

The term mineralization refers to the conversion of organically bound nutrients such as nitrogen (N) and phosphorus (P) to soluble or available forms able to be absorbed by plants (eg. NH_4^+, $H_2PO_4^-$, HPO_4^- etc.) (Paul & Clark 1989). The reverse of this process is called immobilisation. During immobilisation inorganic nitrogen and phosphorus are converted back to organic forms. Both are microbial processes. Factors like soil moisture, temperature, pH, soil micro-organisms (type and population), total nutrient (N or P) and substrate composition affect mineralization. The effects of these factors on mineralization of nitrogen (N) and mineralization of phosphorus (P) are discussed separately.

1.4.3.4.1 Mineralization of nitrogen (N)

The product of mineralization of organically bound nitrogen compounds is ammonium ion (NH_4^+). This process is catalysed by two enzymes : peptidase and proteinase. The mineralization of nitrogenous compounds is affected by the following factors (Black 1968) :

(1) Total nitrogen (N) levels - The net quantity of mineralized nitrogen is approximately proportional to the total quantity of nitrogen in the soil.

(2) Substrate composition, as follows -

(a) Adding protenaceous materials increases mineralization. The microbial population increases rapidly soon after adding protein to the soil. When the amount of protein becomes limiting in the soil, mineral nitrogen can subsequently undergo a secondary surge in levels because nitrogen can be released as the large number of micro-organisms die off.

(b) When sugar is added to soil the opposite results can be observed. The mineral nitrogen levels in soil has been observed to decrease soon after sugar was added (Black 1968). This was attribute to the immobilisation of nitrogen by the rapid growth of micro-organisms. When the sugar content was exhausted, the mineral nitrogen content gradually increased as a result of micro-organisms death and decomposition.

(3) Soil pH - Higher mineralization of nitrogen has been observed when pH is increased in initially acidic soils.

(4) Moisture content : Anaerobic (water logged) condition increases mineralization due to favourable condition for growth of anaerobic mineralising bacteria.

(5) Temperature : The maximum mineralization of nitrogen has been observed to occur in soils at 60°C - 70°C.

1.4.3.4.2 Mineralization of phosphorus

Mineralization of organic phosphorus contributes to the supply of labile inorganic phosphorus in the soil. The enzyme phosphatase is largely responsible for this decomposition process. This enzyme is produced by bacteria and other soil organisms and is also produced by plant roots (Miller & Danahue 1995). As for mineralization of nitrogen, mineralization of phosphorus is also increased by favourable microclimatic conditions. Halstead *et al.* (1963) and Kaila (1965) found that mineralization of phosphorus increases as pH increases. The substrate composition also affects the rate of mineralization of organic phosphorus. Miller and Danahue (1995) found that when the C : P ratio in the soil is about 200 : 1 or lower, phosphorus is readily released by mineralization, but if the C : P ratio is wider (300 : 1 or greater) the micro-organisms use most of the available phosphorus for their own growth and therefore immobilise it.

1.5 Study sites

1.5.1 Derrimut grassland reserve

The Derrimut grassland reserve is situated 15 km west of Melbourne on the eastern end of the extensive Basalt plains of western Victoria. The extent of the reserve is 154 hectares. The mean annual rainfall in this area is 568 mm, distributed fairly evenly throughout the year. The highest monthly mean temperature is 26°C in January and the lowest is 5°C. The highest and lowest recorded temperature in this area during study period were 42°C (20th January 1997) and - 3.2°C (5th of August 1997) respectively. The highest recorded rainfall in the area during the period of study was 31.8 mm on 28th February 1996 (Bureau of Meteorology, Melbourne).

The topography of the reserve and the surrounding area is flat to gently undulating, from 29 m to 36 m elevation above sea level.

The land on which reserve is located was owned by private families until the land was bought by state government in 1985. Since then the Derrimut grassland has been reserved and management action launched. Management action has included prescribed burning in 1988, 1989 and 1992, and weed control in 1992 on a burnt area and heavily weed infested margins (Craigie & Stuwe 1992).

Prior to European settlement the area was typical western Basalt plains grassland, dominated by *Themeda triandra* in all well drained areas. Many species have presumably been depleted or eliminated by continual grazing or lack of proper management practices. Lunt (1990 a) divided the whole area into two vegetation types, wetland and dry grassland. The experimental plots described in this thesis were set up in the dry grassland vegetation type. Dry grassland comprises two grassland types : native dominated *Themeda* grassland and exotic dominated *Vulpia* grassland. Experimental plots were located within *Themeda* grassland. This grassland is the most widespread vegetation in the reserve and occurs in well drained areas that were neither ploughed last century nor grazed heavily this century. The other native species present in this area include *Chrysocephalum apiculatum* (Common Everlasting) and *Leptorhynchos squamatus* (Scaly Button).

The other type of grassland vegetation is *Vulpia* grassland, which is dominated by exotic annual grass, *Vulpia bromides* (Squirrel - tail Fescue) and with sub-dominant native *Stipa bigeniculata* (Kneed Spear - grass).

1.5.2 Laverton grassland reserve

The Laverton grassland reserve is situated 15 km west of Melbourne, on the eastern end of the extensive Basalt Plains of western Victoria. The area of the reserve is 52.6 hectares (Craigie & Stuwe 1992). The mean annual rainfall at Laverton is 568 mm, distributed fairly evenly throughout the year, but it is slightly heavier in spring. The mean maximum temperature is 26°C in January and the mean minimum is 5°C in July.

The topography of the reserve is mostly flat, less than 20 m elevation above sea level. The Olivine basalt underlying the reserve, belonging to the "Newer Volcanics", was laid down during the early Pleistocene, from about five million years ago. The top soils are deeply cracking shallow volcanic clay and clay loam (Craigie & Stuwe 1992).

The land on which the reserve is located was owned by private owner, and in 1911 the land was purchased by the Victorian State Public Works Department. The land was heavily grazed by sheep, cattle and horses until the land was temporally reserved for preservation of native grassland in 1983. The site was probably rarely burnt. No ploughing or cropping took place and there are no records of superphosphate being applied (Craigie & Stuwe 1992).

Prior to European settlement, the area was dominated by *Themeda triandra* grass and with a high species composition of forbs. Since then, many species have presumably been depleted, eliminated or invaded by weeds, as a result of continual or periodic heavy grazing and other improper management practices.

In the most comprehensive floristic survey in 1980, 211 vascular plants were recorded in the reserve. This includes 123 native species (58%) and 88 exotics (42%). The vegetation of the area is divided into two main types, *Themeda* tussock grassland and wetland vegetation. Most of the reserve is *Themeda* tussock grassland, dominated by *Themeda triandra* (Kangaroo Grass), which generally exceeds 90% cover, but in some parts *Danthonia duttoniaana* (Brown-back Wallaby Grass) are common or dominant. Exotic annual grasses such as *Briza minor* (Quaking grass) are abundant throughout the reserve (Craigie & Stuwe 1992).

1.5.3. Victoria University of Technology, St. Albans campus grassland reserve.

This grassland reserve is located on the Keilor plains, approximately about 17 km north west of Melbourne, and at 60 m above sea-level. The extent of the St. Albans campus grassland reserve is about 2.5 ha. The other research sites, Derrimut grassland reserve is situated 5.5 km south of St. Albans site and Laverton grassland is situated 6.5 km south of St. Albans site.

St. Albans campus grassland is dominated by *Themeda triandra* (Kangaroo Grass) with a co - dominant alien grass species *Nassella trichotoma* (Serrated Tussock). The reserve has a clear line between the *Nassella trichotoma* and *Themeda triandra* dominated areas. *N. trichotoma* is dominant on disturbed areas of the site which covers about 45 % of the reserve, and the balance consists of native *T. triandra* grass. The experimental plots described in this thesis were set up in *Themeda triandra* dominated area.

Chapter 2: Measuring nutrient levels in ecosystems

2.1 Introduction

2.1.1 Soil nutrients

There are several ways of expressing the levels of nutrients in the soil. These include : total nutrients, available nutrients, extractable nutrients and labile nutrients. For this study I chose to measure total soil nutrients and extractable soil nutrients in native grasslands. The levels of extractable nutrients were taken as an estimate of available nutrients. The reasons for this are outlined below. The rates and extent of mineralization of nitrogen (N) and phosphorus (P) in soil samples was also studied to determine the extent to which nitrogen (N) and phosphorus (P) can become easily available as a result of decomposition of organic materials in the soil.

2.1.1.1 Total nutrients

The total nitrogen (N) in the soil can be analysed in either of two ways ; the Kjeldhal method and the Dumas (Bremner & Mulvaney 1982). Both methods involve the conversion of organic nitrogen to inorganic form of nitrogen, either the Kjeldhal – ammonium (NH_4^+) form or the Dumas - nitrogen gas (N_2). For this study, total nutrients were analysed only at the start (April 1996) and at the end (April 1998) of field experiments (see details below).

The analysis of total phosphorus (P) and total potassium (K) also requires conversion of insoluble and unavailable form of phosphorus (P) and potassium (K) to the soluble forms PO_4^{3-} and K^+ respectively (Jackson 1958; Allen *et al.* 1972). The total amount of phosphorus (P) in the soil is analysed by digesting soil with $HClO_4$ or fusion with Na_2CO_3. After digesting, the soluble form of phosphorus (PO_4^{3-}) is analysed by colorimetry (Olsen & Sommers 1982).

The total amount of potassium (K) in the soil is analysed by digesting soil with a combination of HF and either H_2SO_4 or $HClO_4$ followed by emission spectroscopy (Olsen & Sommers 1982; Jackson 1958).

2.1.1.2 Available nutrients

The other form of nutrients I will discuss in this chapter and through out my thesis is available nutrients; in particular nitrogen (N), phosphorus (P) and potassium (K) in the soil. The levels of available nutrients (see chapter 1) are usually closely related to the levels of extractable nutrients (see chapter 1). Extractable nutrients are, the ionic form of nutrients in the soil (eg. NO_3^-, NH_4^+, NO_2^-, PO_4^{3-}, K^+) which can be extracted by suitable extractants (see later in this chapter). Some ecologists refer to these as "inorganic or exchangeable" forms of nutrients. Blinkey and Hart (1989) and Schlesinger (1996) found that extractable nutrients provided a reliable index for available nutrients in the soil, when analysed in the each of the seasons.

The predominant forms of extractable nitrogen are NH_4^+ - N and NO_3^- - N. Nitrite (NO_2^-) is seldom present in detectable amounts in most soils (Keeney & Nelson 1982).

There are several methods for estimating available phosphorus (P) from the soil using extractive methods. The method used is dependent on the type of soil. Bray and Kurtz (1945) used HCl/NH_4F for acidic soils. Thompson *et al.* (1960) used water for acidic soils and Olsen and Sommers (1982) used 0.5M $NaHCO_3$ at pH 8.5 for neutral or alkaline soils. Most soil analytical laboratories in Victoria (e.g. State Chemistry Laboratory) use 0.5M $NaHCO_3$ as the extractant.

Available potassium (K) is extracted by a range of methods. Different extracting solutions are used for each of the different methods. The amount of potassium (K) extracted from the soil depends on the nature of the extracting solution

(Knudsen *et al.* 1982). Solutions that have been used to extract potassium (K) include 0.02M HCl, 0.05M HCl, 1M CH_3COONH_4 at pH 7 and 0.5M CH_3COONH_4 (Rayment & Higginson 1992). Soil analytical laboratories in Victoria use 0.05M HCl (Raymont & Higginson 1992).

After extracting extractable nutrients (NH_4^+, NO_3^-, PO_4^{3-} and K^+) using a suitable extractant, the next step is to determine the concentration of the ions in the extract. The method used to determine ionic concentrations should be very sensitive at low ionic concentrations and should not be affected by other ionic species in the extract.

2.1.1.3 Mineralization

Mineralization of nitrogen (N) and phosphorus (P) can be assessed in two ways. These are *in-vitro* mineralization (laboratory incubation) and *in-situ* mineralization (field incubation).

In-vitro **(laboratory incubation) mineralization**

Laboratory incubation for mineralization can be carried out in each of two ways: aerobic incubation and anaerobic incubation. Aerobic incubation is the most frequently used method in which soil samples are held in controlled aerobic environmental conditions. Anaerobic method involves incubation of soil sample under waterlogged closed containers.

Stanford and Epstein (1974) found that the moisture content of soils during incubation affected net nitrogen (N) mineralization. Lamb (1980) and Matson and Vitousek (1981) have demonstrated that the *in-vitro* aerobic method does not reflect field estimates of mineralization. This is because the environmental parameters of the soil are different in different conditions.

Anaerobic methods involve the incubation of soil sample under waterlogged conditions in closed containers. In this method, only the net change in the NH_4^+ is assessed, because no nitrification occurs under anaerobic condition (Blinkey & Hart 1989). The anaerobic method has several advantages. Soil moisture levels are not limiting. All of the nitrogen (N) converted to NH_4^+ can be easily captured and measured. Finally, while the rate of mineralization might not reflect *in-situ* levels, the overall level of potentially mineralizable nitrogen is accurately estimated.

***In-situ* (field incubation) mineralization**

Several methods have been introduced to study field condition mineralization. They include: ion exchange resin bag method (Gibson *et al.* 1985), buried bag method (Eno 1960) and closed top tube incubation (Adams & Attiwill 1986a, 1986b; Raison *et al.* 1987). Raison *et al.* (1989) proposed that the closed top tube (PVC) incubation method causes less disturbance to the natural processes and it provides reliable quantitative estimates of fluxes of mineral nitrogen (N) in the soil. The closed top tube (PVC) was chosen in my study and a detailed description of the method is outlined in chapter four.

2.1.2 Other related soil ecological parameters

2.1.2.1 Organic carbon

There are several ways of expressing the level of carbon (C) in the soil. These are: total carbon , oxidisable carbon, organic matter and organic carbon. In my study organic carbon was chosen as the most useful and reliable form of soil carbon to investigate. Organic carbon provides an estimate of the biomass of oxidisable organic material (living and dead roots and micro-organisms) in the soil.

Organic matter and organic carbon can be determined in a number of different ways. One method involves measuring the amount of CO_2 released following exposure of soil to ignition condition. Another method involving "loss in conversion" measures the difference in weight of a soil sample before and after high temperature ignition and loss to gaseous CO_2 of organic carbon (Allen *et al.* 1972). The method of "loss in conversion" was used in my experiments to determine organic carbon content in the soil.

2.1.2.2 Soil pH

pH of soil (chapter 1) can be determined by two methods, colorimetric and electrometric (Allen *et al.* 1972). The colorimetric method is not precise and accurate and is used only for rough field tests (Allen *et al.* 1972; Jackson 1958). The electrometric method (pH meter) is more accurate and is mostly used in ecological studies. This was the method used in my investigations.

2.1.2.3 Soil moisture

Soil moisture content can be determined by several techniques. The gravimetric method, otherwise known as thermal drying at 105°C to a constant weight is the most common technique (Rayment & Higginson 1992). There are two ways of expressing moisture in the soil, the fresh (field condition) moisture content and the air dried moisture content. In air dried method soil is air-dried prior to soil moisture analysis. Fresh moisture content was chosen in my study because it was more accurately as an estimate of soil moisture content under field conditions.

2.1.3 Soil sampling methods

Several factors need to be considered when taking soil samples in the field. These are: number of replicates per treatment, soil depth, transport and storage. In the field, a high degree of spatial variability in soil conditions may exist over a small area. Increasing the number of cores increasingly allows the variability of the soil characteristics to be taken into account in subsequent statistical analysis (Allen *et al.* 1972). Jackson (1958) points out that the number of cores needed depends on the uniformity of the soil profile. Uniformity varies with touched (disturbed or fertilized) or untouched soils. Intact native grassland soils tend to be untouched and the soil profile is relatively uniform. Adams *et al.* (1989) found that the levels of nutrients in forests changed with the soil depth. Soil nutrient levels are usually highest at the soil surface and decrease rapidly with depth (Charley & West 1977; Burke 1989; Evans & Ehleringer 1993). The recommended depth of sampling varies with the plant species and communities under study (Keeny & Nelson 1992).

2.1.4 Storage of soil samples prior to analysis

Pretreating soil samples in the laboratory before analysis involves drying, sieving, mixing and storing. Drying is not suitable for some analysis because of the rapid changes that takes place in the status of some ionic species (eg. ammonium (NH_4^+) ion oxidises to nitrite (NO_2^-) and nitrate (NO_3^-) ions). Therefore many types of analysis are carried out on moist samples immediately after collection (eg. extractable nitrate - nitrogen (NO_3^- - N), ammonium - nitrogen (NH_4^+ - N), acid extractable phosphorus (P) and extractable potassium (K) (Jackson 1958), or a short cold storage is acceptable (Allen *et al.* 1972; Adams *et al.* 1989; Keeny & Nelson 1982).

2.1.5 Statistical analysis

The variation of analytical results from the mean value is an important parameter to consider when determining the precision of results and making comparisons between treatments (Allan *et al.* 1972; Zar 1996). This variance can be expressed in two related ways, *variance of means* and *standard error.*

When comparing the effects of different experimental treatments the significance in difference between means of replicates from sample populations can be analysed by a variety of statistical methods. One such method is analysis of variance (ANOVA - single factor). While ANOVA is a powerful comparative tool, one limitation of the method is that it does not compare mean differences of sample populations individually. To overcome this problem multiple comparison tests have been devised. Among the most widely accepted and commonly used methods are Tukeys' test (also known as honestly significant difference test and wholly significant difference test) and Newman-Keuls' test (Zar 1996). These and other appropriate statistical tests were used to compare results of various treatments on soil nutrient levels. Details of statistical analysis are outlined in appropriate sections of the thesis.

2.2 Devising and testing a standard methods

Testing for some soil parameters needed to be tested and modified to suit Basalt clay soils. The methods outlined in the following section were those developed for the analysis of soil nutrient levels and related parameters in Basaltic clay soils under lowland grassland remnants in the Keilor Plains of Melbourne. The preliminary methodical studies to find out the most effective ways of determining soil nutrient levels were performed at Victoria University of Technology (VUT), St. Albans grassland reserve (on the southern side of St. Albans campus).

2.2.1 Soil sampling

Two soil cores (2.5 cm diameter steel soil core) were taken at 2 cm - 5 cm soil depth from each replicate plot for analysis. This specific depth was chosen because in grasslands 60% of underground biomass is present at 0 - 5 cm soil depth and seasonal fluctuation of nutrients occurs mostly in this zone (Dormaar 1992). The first two centimeters of soil were excluded so as to remove influences of the leaf litter layer. The Moist soil cores from each plot were derooted, destoned and thoroughly mixed together to form one bulk sample. These samples were stored at 4°C until further analysis could be carried out (Adams *et al.* 1989).

2.2.2 Methods used for soil analysis

2.2.2.1 soil moisture

Soil cores from the field were immediately sealed in plastic pre-weighed containers and stored at around 4°C to prevent any water loss due to evaporation. The full wet weight of the soil core (with container) was recorded and the soil core was carefully transferred to a dry pre-weighed porcelain crucible. The rocks and roots were removed from the core and fresh weight of roots was recorded. Rocks were transferred back to the vial and a small amount of deionised water was added to wash off any soil still in the vial and rocks. This water was added to the soil sample in the crucible.

The crucible with soil and rocks were separately placed in an air circulation oven and dried at 105°C to a constant weight.

Calculations :

fresh (initial) mass of soil = mass of soil with vial - (mass of empty vial + dry mass of rocks + fresh mass of roots)

dry mass of soil = mass of dried soil with crucible - mass of crucible

$$\text{moisture content (\%)} = \frac{\text{initial mass of soil} - \text{dry mass of soil}}{\text{initial mass of soil}} \times 100$$

The extractable nutrients NH_4^+ - N, NO_3^- - N, PO_4^{3-} - P and K, as well as organic carbon were derived from fresh soil samples. However final levels of these parameters were expressed in units per gram dry weight of soil. It was therefore necessary to estimate the dry weight equivalent of fresh (ie. moisture containing) samples of soil, using the formula.

$$\text{estimated dry weight of soil (g)} = \frac{\text{fresh weight of soil (g)} \times (100 - \text{moisture content})}{100}$$

2.2.2.2 Organic carbon (C)

Organic carbon content was determined via the "loss on ignition" method. About 1g of soil was accurately weighed in a dry (mass known) porcelain crucible. Two drops of H_2O_2 (35%) was added to the soil (to promote oxidation of organic components) and the crucible with soil was placed in a muffle furnace. The temperature of the muffle furnace was adjusted to 450°C. When the temperature of the muffle furnace reached 450°C, the crucible with soil sample was allowed to remain at this temperature for four hours (Allen *et al.* 1972). The muffle furnace was then turned off and the crucible with soil was left to cool. The crucible was transferred to a desiccator and cooled to room temperature. The percentage of "loss on ignition" was calculated as follows.

$$\text{loss on ignition (\%)} = \frac{\text{the mass loss after ignition}}{\text{mass of oven dried soil}} \times 100$$

The constituents of organic matter (plants animal and micro-organism matter) are different from one soil to another. Therefore, the conversion of loss on ignition (organic matter) to organic carbon depends on the type of soil. A widely accepted conversion factor for most soil types is to assume that organic matter contains 58% carbon (Allen *et al.* 1972).

$$\text{organic carbon (C)} = \text{loss on ignition} \times 0.58$$

Sample calculation :

The following steps in columns were followed when organic carbon (C) content was calculated during the methodical study at St. Albans grassland site.

Table 2.1: Results and analysis of soil organic carbon

sample	oven dry mass of soil (g)	mass of crucible (g)	crucible+soil after drying (g)	mass of soil after drying (g)	loss on ignition (%)	org. C (%)
1	0.8897	53.2621	54.0850	0.8229	7.51	4.36
2	0.8623	53.4373	54.2302	0.7929	8.04	4.66
3	0.8672	52.8717	53.6773	0.8056	7.10	4.12
4	0.9808	53.4885	54.3986	0.9101	7.21	4.18

2.2.2.3 pH

The method used follows that described by Rayment and Higginson (1992).

About 5 g of air dried soil was accurately weighed in a 50 mL screw cap glass bottle.

A 25 .0 mL volume of deionised water was added and the vessel was closed. The bottle was shaken on an end - over - end mechanical shaker (Griffin flask shaker) for one hour. The suspension was allowed to settle for 30 minutes.

The pH of the suspension was measured using a pH meter (ORION - SA520) precalibrated with buffer solutions of pH 4 and 7. During pH measurements, the electrodes of the pH meter were well immersed in the soil suspension and the pH value was recorded when the reading was stable.

2.2.2.4 Determining available soil nutrients

2.2.2.4.1 Extractable nitrogen (N)

Extraction of NH_4^+, NO_3^- and NO_2^-

The methods used in this study have been described by Adams *et al.* 1992. About 5 g of fresh soil was accurately weighed in a 250 mL conical flask and shaken with 50 mL 1M KCl using a reciprocal shaker for 45 minutes. The extract was allowed to settle overnight at 4°C. Three controls, containing only 50 mL of 1M KCl were included in every experiment. Three spiked soil samples containing 0.2 mg L^{-1} of each NH_4^+ - N , NO_3^- - N and NO_2^- - N were also included. Control solutions and spiked samples were subjected to the same extraction procedure as other samples.

Each extract was centrifuged and the supernant layer was carefully separated by filtering into a separate container. This supernant layer was divided into two halves for further analysis. One half was used to analyse extractable NH_4^+ and the other was used analyse extractable NO_3^- and NO_2^-.

Determination of NH_4^+ in the extract

Three methods were initially investigated to determine the optimum method for estimating NH_4^+ in the basalt clay soil extract.

1. Ion exchange chromatography

This method was not successful because highly concentrated K^+ in the solution (from the extraction medium) interfered with analysis. No accurate and reproducible method for estimating NH_4^+ in the extract was possible using this method.

2. Flow injection auto analyser

This method did not give good recovery with spiked samples. This may be due to interference with other ions present in the solution.

3. The method described by Strictland and Parsons (1977)

The basis of this method is NH_4^+ that present in the solution is oxidised by NaOCl and then coupled with phenol to produce a blue colour (indophenol).

This method gave satisfactory and reproducible recovery percentages from spiked samples and reliable estimates of NH_4^+ levels compared to the soil from other grassland ecosystems.

The following reagents were required to analyse ammonium (NH_4^+) ion in the solution using this method.

Reagents :

phenol - alcohol solution - 10% (w/v) phenol in ethanol (95% v/v).

Sodium nitroprusside solution - 0.5% (w/v) sodium nitroprusside in water.

Alkaline - citrate solution - aqueous solution containing mixture of 20% (w/v) trisodium citrate ($C_3H_4OH(COONa)_3.H_2O$) and 1% (w/v) sodium hydroxide (NaOH) in deionised water.

Oxidising solution - mixture of alkaline citrate solution and sodium hypochlorite (laboratory grade) in 4:1 volumes.

Standard solution of ammonium chloride (NH_4Cl) - a stock solution containing 100 mg L^{-1} of NH_4^+ - N (0.3821g L^{-1} of aqueous NH_4Cl). A range of standard solutions 0.10 mg L^{-1}, 0.25 mg L^{-1}, 0.50 mg L^{-1} and 1.00 mg L^{-1} in 1M KCl were prepared by dilution this solution.

Procedure :

2.50 mL of KCl soil extract was diluted to 5.00 mL. The following solutions were subsequently added to each 5.00 mL of the diluted extract.

0.20 mL of phenol - ethanol solution
0.20 mL of sodium nitroprusside solution
1.00 mL of oxidising solution

After adding the above solutions, the contents were thoroughly mixed and left for one hour at 22°C - 27°C to develop the blue colour (indophenol). The absorbance of the blue colour was measured at 640 nm in a 10 mm pathway cuvette using UV-Visible spectrophotometer (Varian CARY 1 - 1990).

At every analysis four standard solutions containing 0.10 mg L^{-1}, 0.25 mg L^{-1}, 0.50 mg L^{-1} and 1.00 mg L^{-1} in 1M KCl were prepared and 5.00 mL of this standard was treated in the same way as soil extracts. The absorbance of the blue colour was measured at 640 nm. A standard curve was constructed by plotting absorbance levels against respective concentrations of NH_4^+ - N in the four standard solutions. The concentrations of NH_4^+ - N in the extracts and controls were calculated using a linear regression equation of the standard curve.

As an example of results obtained during analysis of Basaltic clay soil the following results were obtained from soil at St. Albans grassland reserve site, during the development of the methodology. The methods for calculating of NH_4^+- N in the soil is also outlined, underneath the set of tables.

Table 2.2: Masses of soil samples used for the determination of NH_4^+ - N and NO_3^- - N in the soil.

sample	fresh mass of soil (g)	moisture contents (%)	oven dry mass of soil (g)
1	5.3221	35.10	3.4540
2	5.0014	27.68	3.6170
3	5.5662	30.23	3.8835
4	5.1536	29.84	3.6157
1sp	5.3211	35.10	3.4533
2sp	5.0501	27.68	3.6522
3sp	5.5632	30.23	3.8814
4sp	5.1446	29.84	3.6094

sp - spiked solutions, containing 0.2 mg L^{-1} of NH_4^+ - N in the soil extract.

The following standard solutions of NH_4^+ - N in 1M KCl were prepared from the stock solution of NH_4Cl. Their absorbances (indophenol blue) at 640 nm with UV spectrometer is given below.

Table 2.3: Standard NH_4^+ - N solutions and absorbances

concentration (mg L^{-1})	absorbance	mean absorbance
0.00	0.0400, 0.0378, 0.0379	0.0385
0.10	0.1122, 0.1121, 0.1121	0.1121
0.25	0.3305, 0.3302, 0.3303	0.3303
0,50	0.5990, 0.5985, 0.5987	0.5987
1.00	1.1895, 1.1896, 1.1898	1.1896

The linear regression equation for the graph is $y = 1.096x + 0.028$

Table 2.4 : Soil analytical results (determination of NH_4^+ - N)

sample	mean absorbane	concentration (2 x diluted) (mg L^{-1})	actual concentration (original - control) (mg L^{-1})	µg of NH_4^+-N in 50 mL	recovery (%) in spiked samples	µg g^{-1} (dry wt.)
control	0.2649	0.2162	0.00	-	-	-
1	0.3549	0.2983	0.1642	8.21	-	2.38
2	0.3589	0.3020	0.1740	8.58	-	2.37
3	0.3534	0.2969	0.1614	8.07	-	2.08
4	0.3472	0.2912	0.1530	7.65	-	2.12
1sp	0.4101	0.3394	-	-	87.5	-
2sp	0.4621	0.3960	-	-	98.0	-
3sp	0.3985	0.3380	-	-	86.0	-
4sp	0.4067	0.3383	-	-	84.0	-

This method produced reliable estimates of extractable NH_4^+- N levels in grasslands compared to other ecosystems and other previous work investigating grassland nutrient levels (see chapter 3). The method also produced high percentage recovery of NH_4^+- N from spiked samples. Therefore, this method was chosen to analyse extractable NH_4^+ - N in the soil.

Sample calculation : The experimental results of sample 1 are used to demonstrate the calculation performed.

$y = 1.096x + 0.028$
y = absorbance
x = concentration (mg L^{-1})

concentration of NH_4^+ - N in sample 1 extract (2 x diluted) $= \dfrac{0.3549 - 0.028}{1.096}$ mg L^{-1}

$= 0.2983$ mg L^{-1}

Actual NH_4^+ - N in sample 1 extract (2 x diluted) = 0.2983 - con. of NH_4^+ -N in control
= 0.2983 - 0.2162 mg L^{-1}
= 0.0821 mg L^{-1}

NH_4^+ - N in the original extract = 0.0821 x 2 mg L^{-1}
= 0.1642 mg L^{-1}

Therefore NH_4^+ - N in 50 mL $= \dfrac{0.1642 \times 50 \times 1000}{1000}$ µg
= 8.21 µg

The amount of extractable NH_4^+ - N in sample 1 $= \dfrac{8.21 (\mu g)}{3.454 \text{ (dry mass of sample 1) (g)}}$

= 2.38 µg g^{-1} (dry mass of soil)

Determination of NO_3^- - N and NO_2^- - N in the soil extract.

Two methods were investigated as potential ways to determine NO_3^- - N and NO_2^- - N in the soil extract.

1. Ion exchange chromatography

This method was unsuccessful because the high concentrations of chloride ion (Cl^-) in the KCl extractant interfered with the separation of NO_3^- and NO_2^- ions.

2. Flow injection auto analyser

This method is described in tecator, ASN 143-02/90

The principle of this method is that NO_3^- in the solution is reduced to NO_2^- by a Cd reductor. Then NO_2^- is reacted with acidic sulphanilamide solution to form diazo compound. Diazo compound is coupled with N- (1- naphtyl) - Ethylene Diamine Dihydrochloride (NED) to form a purple azo dye. This azo dye is measured at 540 nm.

The concentration of NO_2^- (already in the sample) is analysed prior to reduction of NO_3^- to NO_2^- . Following this, the NO_3^- is reduced and the resulting concentration of NO_3^- is used to calculate the original levels of NO_3^- + NO_2^-. By subtracting the concentration of NO_2^- (already in the sample) from the total NO_3^- + NO_2^- the concentration of NO_3^- in the sample can be calculated.

Analysis of NO_2^- - N

Reagents required :

a. ammonium chloride (NH_4Cl) solution- 5.2 g of NH_4Cl was dissolved in 110 mL of deionised water.

b. sulphanilamide solution ($C_6H_8N_2O_2S$)- 2.0 g of sulphanilamide was dissolved in 100 mL of deionised water and mixed with 10 mL of concentrated HCl.

c. N- (1- napthyl) - Ethylene Diamine Dihydrochloride solution (NED) - 0.2 g of NED was dissolved in 110 mL of deionised water.

d. standard NO_2^- N solution - standard stock solution containing 200 mg L^{-1} NO_2^- - N was prepared by dissolving 0.2464 g of sodium nitrite (NaNO2) in 250 mL deionised water in 250 mL volumetric flask. A range of standard solutions 0.10 mg L^{-1}, 0.25 mg L^{-1}, 0.50 mg L^{-1} and 1.00 mg L^{-1} in IM KCl were prepared by diluting a suitable volume of the stock solution with deionised water.

Procedure

The instrument was calibrated with standard solutions first. Then the soil extracts were injected as explained in the operating manual.

Analysis of NO_3^- - N

reagents required

The same reagents as above (a, b and c) were required as NO_2^- - N analysis.

d. standard NO_3^- -N solution - standard stock solution containing 200 mg L^{-1} of NO_3^- - N prepared by dissolving 0.1214 g of analytical grade $NaNO_3$ with deionised water in 100 mL volumetric flask and added water upto the mark. This solution was initially diluted by 20 times and made 10 mg L^{-1} solution. This solution was used to prepare a range of standard solutions of NO_3^- - N, comprising 0.0 mg L^{-1}, 0.1 mg L^{-1}, 0.5 mg L^{-1}, 1.0 mg L^{-1} and 2.0 mg L^{-1} in IM KCl.

Procedure :

The flow injection analyser was calibrated with standard solutions. Then the soil extracts were injected into the instrument as explained in the operation manual. As an example of the outcomes of analysis following results were obtained from soil from Victoria University St. Albans grassland reserve site.

Table 2.5 : Soil analytical results (determination of NO_3^- - N)

sample	average concentration of NO_3^- - N (mg L^{-1})	actual concentration (average concentration - control) (mg L^{-1})	NO_3^- - N (µg) in 50 mL	recovery (%) in spiked sample	NO_3^- - N (µg g^{-1}) (dry mass)
control	0.0564	0.0000	0.00	-	-
1	0.1280	0.0716	3.58	-	1.04
2	0.1039	0.0475	2.38	-	0.66
3	0.1085	0.0521	2.61	-	0.67
4	0.0985	0.0421	2.11	-	0.58
1sp	0.3031	0.2467	-	90.83	-
2sp	0.2753	0.2189	-	88.44	-
3sp	0.2995	0.2431	-	96.42	-
4sp	0.2309	0.1972	-	81.45	-

sp - spiked soil samples.

Note : No detectable levels of NO_2^- - N were found in any of the samples assessed. The above results therefore equal to concentration of NO_3^- - N in the samples.

* This was found to be the case for all KCl soil extracts tested (the NO_2^- - N assessment prior to NO_3^- - N assessment was not continued).

<u>Calculation</u> :

Analysis of sample 1 is used to explain calculation performed.

Actual concentration of NO_3^- - N in the soil extract = 0.1280 - 0.0564 mg L^{-1}
= 0.0716 mg L^{-1}

The amount of NO_3^- - N in 50 mL extract = $\frac{0.016}{1000} \times 50 \times 1000$ ug
= 3.58 μg

The oven dry mass of soil = 3.4540 g

The extractable NO_3^- - N in sample one soil = $\frac{3.58\ (\mu g)}{3.454\ (g)}$
= 1.04 μg g^{-1} (oven dry mass)

This method produced reliable ranges of available NO_3^- - N in grassland soil samples (chapter 3). High recovery percentages were obtained with spiked soil samples.

2.2.2.4.2 Extractable phosphorus (P)

Extraction of PO_4^{3-} - P

The method used has been described by Olsen and Sommers (1982). About 5 g of fresh soil was accurately weighed in a 250 mL conical flask and shaken with 100 mL of 0.5 M $NaHCO_3$ at pH 8.5 on a reciprocal shaker for 30 minutes. Each flask was given a final shake immediately before pouring into a centrifuged vial. The suspension was centrifuged at 3000 rpm for 5 minutes to obtain a clear solution.

Three controls (containing only 0.5 M $NaHCO_3$ at pH 8.5) and three spiked soil sampled were also included. Spiked soil samples were prepared by adding 0.5 mL of KH_2PO_4 solution containing 100 mg L^{-1} of P (or 0.4393 g L^{-1} of KH_2PO_4) to the soil slurry. Control and spiked samples were subjected to the same procedure for extraction and analysis as for field samples.

Determination of P in the extract

Several methods of analysis were investigated.

1. Flow injection auto analysis.

The method is described in the manual for tecator, ASN 146-01 / 90. This method was unsuccessful for several reasons, including : results not reproducible; negative absorbances obtained and no good recovery percentage with spiked soil samples. These problems may have arisen because of interference by other ionic species present in the mixture.

2. The manual colorimetric method described by Olsen and Sommers (1982).

Reagents required :

a. Ammonium paramolibdate ($(NH_4)_6Mo_7O_{24}.4H_20$) solution - 1.5 g of ammonium paramolibdate was dissolved in about 30 mL of deionised water. A 0.036 g of potassium antimony tartarate ($KSbO.C_4H_4O_6$) was dissolved in 12.5 mL of deionised water. Separately prepared solutions of these compounds were added to 125 mL of 2.5 M H_2SO_4 in 250 mL volumetric flask. The contents were mixed thoroughly and diluted to 250 mL by adding deionised water. This solution was labelled as A.

b. Ascorbic acid solution - 1.32 g of ascorbic acid was dissolved in 250 mL of reagent A. This solution was labelled as B.

c. H_2SO_4 solution - 0.5 M H_2SO_4 solution

d. Stock standard phosphate solution - 0.1098 g of potassium dihydrogen phosphate (KH_2PO_4) was dissolved in deionised water in 250 mL volumetric flask and adjusted the volume up to the mark (250 mL) by adding deionised water. This solution contains 0.1 mg L^{-1} of P. A series of standards of phosphorus (P), 0.00 mg L^{-1}, 0.1 mg L^{-1}, 0.25 mg L^{-1}, 0.50 mg L^{-1}, 1.00 mg L^{-1} in 0.5M $NaHCO_3$ was prepared by diluting a suitable volume from stock standard solution with deionised water.

Procedure:

Ten millilitres of the extract was placed in 50 mL volumetric flask and acidified with 2.5 M H_2SO_4 to pH 5. This was carried out by taking ten millilitres of the extract to another beaker and determined the amount of 2.5 M H_2SO_4 required to bring the solution to pH 5. Then the same amount of 2.5 M H_2SO_4 was added to all the volumetric flasks containing soil extract. The flasks were left for about ten minutes until no more gas CO_2 gas evolved from the solution. Deionised water (30 mL) was added to each flask and was shaken well to mix the contents properly. Then eight millilitres of reagent B was added to each flask using an auto pipette. Deionised water was added upto the mark to each flask to make the final volume to 50 mL. The contents in the flask were mixed properly and left for ten minutes until a blue colour appeared.

The same procedure was followed to each standard of ten millilitres contained in separate 50 mL volumetric flasks.

The absorbance of blue colour was measured at 880 nm using UV-Visible spectrophotometer (Varian CARY 1 - 1990).

As an example of the outcomes of this analysis, the following results were obtained from soil samples taken from Victoria University of Technology, St. Albans campus grassland reserve site.

Table 2.6: The masses of soil samples used for the determination of extractable P in the soil.

sample	fresh mass of soil (g)	moisture content (%)	oven dry mass of soil (g)
1	5.5883	35.10	3.3023
2	5.3462	27.68	3.8663
3	5.4479	30.23	3.8009
4	5.0526	29.84	3.5449
1sp	5.5921	35.10	3.3047
2sp	5.3447	27.68	3.8653
3sp	5.4052	30.23	3.7712
4sp	5.0897	29.84	3.5709

sp - spiked samples

Table 2.7 : Standard solutions and absorbances

concentration (mg L^{-1})	average absorbance
0.00	0.0054
0.10	0.0235
0.25	0.0357
0.50	0.0861
1.00	0.1626

The linear regression equation is $y = 0.142x + 0.004$

y = absorbance

x = concentration (mg L^{-1})

Table 2.8 : Analytical results - determination of PO_4^{3-} - P in the soil

sample	average absorbance	concentration (mg L^{-1})	actual concentration (mg L^{-1})	µg in 100 mL	recovery (%) in spiked samples	available P (µg g^{-1}) (dry mass)
control	0.0093	0.0373	0.0000	-	-	-
1	0.0197	0.1106	0.0733	7.33	-	2.21
2	0.0172	0.0930	0.0557	5.57	-	1.44
3	0.0175	0.0951	0.0578	5.78	-	1.52
4	0.0145	0.0739	0.0366	3.66	-	1.03
1sp	0.0681	0.4514	0.4141	41.41	72.23	-
2sp	0.0863	0.5796	0.5423	54.23	97.58	-
3sp	0.0837	0.5613	0.5240	52.40	93.94	-
4sp	0.0782	0.5225	0.4852	48.52	90.42	-

sp - spiked samples.

This method was chosen for analysis of extractable phosphorus in the soil as it gave very high recovery percentages with spiked samples. At every analysis fresh standards were prepared, calibrated and determined for phosphorus (P) concentration in the sample using linear regression equation.

Sample calculation :

The experimental results of sample one is used to explain the method of calculation.

The actual concentration of PO_4^{3-} - P in sample one = 0.1106 - 0.0373 mg L^{-1}

= 0.0733 mg L^{-1}

The amount of P in 100 mL $= \frac{0.0733 \times 100 \times 1000}{1000}$ µg

= 7.33 µg

oven dry mass of soil = 3.3023 g

The amount of extractable P in the soil $= \frac{7.33\ (\mu g)}{3.3023\ (g)}$

= 2.21 µg g^{-1} (dry mass of soil)

The same method of calculation was performed for every soil sample.

2.2.2.4.3 Extractable potassium (K)

Extraction of available K

The method used has been described by Skene (1956). About 5 g of soil was accurately weighed in a 250 mL conical flask and shaken with 100 mL of 0.05 M HCl with reciprocal shaker for one hour. The extract was allowed to stand overnight to settle the supernant. Three controls containing only 100 mL of 0.05 M KCl and three spiked soil samples were also included and followed the same procedure as other soil samples. Spiked samples were prepared by adding one millilitre of 500 mg L^{-1} of K^+ solution to the soil sample.

The supernant was carefully transferred to a centrifuged vial and centrifuged for five minutes.

Determination of K^+ in the extract

Reagents required :

a. CsCl solution - 10 g L^{-1} of Cs (12.67 g L^{-1} of CsCl) solution.

b. stock standard solution of K^+- 1 g L^{-1} of K^+ stock solution. This was prepared by dissolving 0.1907 g of analytical grade potassium chloride (KCl) in 100 mL deionised water. The standard solutions of 0.00 mg L^{-1}, 0.20 mg L^{-1}, 0.50 mg L^{-1}, 1.00 mg L^{-1}, 1.50 mg L^{-1} and 2.00 mg L^{-1} of K^+ in 1g L^{-1} of Cs and in 0.005M HCl (ten times diluted of 0.05 M HCl) were prepared by diluting suitable volumes of stock standard solutions.

10 mL of the clear supernant was placed in a 100 mL volumetric flask and 10 mL of 10g L^{-1} of Cs solution (as CsCl) was added. The volume in the flask was adjusted to 100 mL by adding deionised water. Cs was added to the solution to suppress the ionisation of K in the air - acetylene flame (Varian A W 39.10 - Atomic Absorption Spectrometry instrument manual, 1988). The concentration of K^+ in the diluted soil extract was determined by atomic absorption spectrometer (Spectra AA - 400, 1988).

The instrument was calibrated with standard solutions before analysing the soil extracts.

The following results were obtained during the development of methods with soil from the Victoria University of Technology, St. Albans campus grassland reserve site.

Table 2.9: Masses of soil samples used for the analysis of extractable potassium (K).

sample	fresh mass of soil (g)	moisture content (%)	dry mass of soil (g)
1	5.1254	35.10	3.3263
2	5.3884	27.68	3.8968
3	5.0512	30.23	3.5242
4	5.1639	29.84	3.6229
1sp	5.1136	35.10	3.3187
2sp	5.3921	27.68	3.8995
3sp	5.0553	30.23	3.5270
4sp	5.1597	29.84	3.6200

sp - spiked soil samples

Table 2.10: Standard solutions and absorbances

standards (mg L^{-1})	mean absorbance
0.00	0.002
0.20	0.067
0.50	0.170
1.00	0.343
1.50	0.514
2.00	0.688

Table 2.11: Analytical results - determination of extractable K

sample	mean absorbance	concentration (x 10 diluted) (mg L^{-1})	original concentration (mg L^{-1})	K^+ in 100 mL (μg)	recovery (%) in spiked samples	K^+ in the soil (μg g^{-1}) (dry mass)
control	0.003	0.00	0.00	0.00	-	
1	0.470	1.35	13.55	1350	-	405.85
2	0.385	1.12	11.20	1120	-	287.41
3	0.488	1.42	14.20	1420	-	402.92
4	0.393	1.14	11.40	1140	-	314.66
1sp	0.612	1.78	17.80	1780	96.21	-
2sp	0.512	1.49	14.90	1490	91.97	-
3sp	0.635	1.85	18.50	1850	96.35	-
4sp	0.546	1.59	15.90	1590	96.95	-

sp - spiked samples

Sample calculation :

The analytical results of sample one is used to explain the method of calculation

concentration of K^+ in ten times diluted sample = 1.35 mg L^{-1}

concentration of original sample (undiluted) = 13.50 mg L^{-1}

The amount of K^+ in 100 mL extract

$$= \frac{13.50}{1000} \times 100 \text{ mg} = 1.35 \text{ mg}$$

The dry mass of soil (table) = 3.3263 g

The amount of extractable K^+ in soil

$$= \frac{1.35 \times 1000\ (\mu g)}{3.3263\ (g)} = 405.85\ \mu g\ g^{-1} \text{ (dry mass of soil)}$$

This method was chosen for analysis of available potassium (K) in the soil as it gave very high recovery percentages with spiked samples.

At every analysis fresh standards of K^+ were prepared and used to calibrate the instrument.

2.2.3 Statistical analysis

The calculations of sample mean, standard error and tests of significance (significant difference of sample means by one way ANOVA) were performed by Microsoft excel, 94 - 96 versions.

Multiple comparisons values with different letters differ significantly ($p < 0.001$, $p < 0.005$, $p < 0.01$) and sharing same letters are not significantly different ($p > 0.01$, $p > 0.05$). Details of statistical analysis are present in appendix 3.

Linear regression curves and equations were obtained from Macintosh, CA - Cricket graph 111 - version 1.5 (1993).

2.3 Summary of standard methods for soil analysis

The methods for soil analysis described below are the methods considered to be the most appropriate for the estimation of extractable nitrogen (N), phosphorus (P) and potassium (K) and other soil ecological parameters in basalt native grassland soil of the Keilor plains. In subsequent chapters these are referred to as the "standard methods". They were the methods used for all soil analysis, unless otherwise specifically stated.

Soil moisture : Fresh soil sample (without roots and rocks) was dried at 105°C to a constant mass. The mass difference between fresh soil and dried soil was used to calculate soil moisture content. See section 2.2.2.1 for details.

Organic carbon (C) : Organic carbon (C) was determined via loss in conversion (Allan *et al.* 1972). According to this method fresh soil was ignited at 450°C. The percentage loss during ignition was used to calculate the amount of carbon in the soil. See section 2.2.2.2 for details.

Soil pH : The method is described Rayment & Higginson (1992). Air dried soil was well mixed with deionised water and pH of the soil -water suspension was measured according to this method. See section 2.2.2.3 for details.

Extractable nitrogen (NH_4^+, NO_3^- and NO_2^-) :

Fresh soil was extracted with 1M KCl as described by Adam *et al.* (1992). See section 2.2.2.4.1 for details.

The NH_4^+ - N in the extract was determined by colorimetry, as described by Stricland and Parsons (1977).

The NO_3^- - N in the extract was determined by the method described in - Tector, ASN 143 - 02/90 (Flow Injection Auto analyser).

Extractable phosphorus (P) :

The method was as described by Olsen and Sommers (1982). Fresh soil was extracted with 0.5 M $NaHCO_3$ at pH 8.5. The PO_4^{3-} - P in the extract was determined by colorimetry. See section 2.2.2.4.2 for details.

Extractable K :

The method used was as described by Skene (1956). Fresh soil was extracted with 0.5 M HCl, and the soil extract was analysed for K^+ by Atomic Absorption Spectrometer. See section 2.2.2.4.3.

Total N, P and K :

Total levels of nitrogen (N), phosphorus (P) and potassium (K) were analysed by State Chemistry Laboratory, Werribee, Victoria, Australia.

Total P and K : Nitric + Perchloric acid digestion followed by analysis on ICP - A6S.

Total N : by Leco N analyser (FP 2000 analyser).

Chapter 3

Levels of available soil nutrients (nitrogen, phosphorus and potassium) and other physico-chemical properties of native grassland soils.

3.1 Introduction

3.1.1 Soil nutrient levels in grasslands and other ecosystems

There is a little published information on nutrient levels or dynamics in soils of native grasslands in Australia. What published data is available is often expressed in a variety of ways (eg. total nutrients, labile nutrients and organic form of nutrients) which makes comparisons difficult. Sometimes brief studies of native grassland soils are included for comparison with forest soils. This introduction summarises the information available from Australian grasslands, forests and related overseas studies of nutrients and associated soil parameters.

A larger number of studies have been carried out on forest soil nutrients and related properties than for grassland soils. Maggs and Hewett (1993) studied organic carbon (C) and nutrients (nitrogen (N), phosphorus (P) and potassium (K)) in surface soils (depth was not mentioned) in primary rain forests , secondary rain forests and in derived grasslands (unmanaged pasture), in two different soil types (Krasozem and Prairie soil), on the Atherton Tableland in North East Queensland. The results of this study are summarised in table 3.1. While the nutrient levels in rainforest soils were generally higher than for grassland soils (with the exception of total phosphorus) the differences were not great.

Table 3.1 Summary of soil nutrient levels and associated parameters measured by Maggs and Hewett (1993) in soils under grasslands and rainforests in Queensland.

soil nutrient	derived grassland	primary rain forest
Total Kjeldhal- N (%)	0.28 - 0.56	0.58 - 0.81
Labile N ($\mu g\ g^{-1}$)	38 - 45	45 - 61
Total P (%)	0.153 - 0.48	0.198 - 0.314
Exchangeable K ($\mu g\ g^{-1}$)	340 - 573	487 - 815
Organic C (%)	5.13 - 8.33	6.71 - 11.08

The soil nutrient levels at 2 cm soil depth in snow- tussock grasslands in New Zealand was studied by Ross *et al.* (1997). The values they obtained are as follows (Table 3. 2).

Table 3. 2. Summary of soil nutrient levels and associated parameters measured by Ross *et al.* (1997) in snow-tussock grasslands in New Zealand.

Extractable N	21 ($\mu g\ g^{-1}$)
Extractable P	11 ($\mu g\ g^{-1}$)
Total N	0.4 (%)
Total C	8.1(%)

Adams *et al.* (1989) studied soils in Northern Tasmania forests. The sites they studied were Saddleback (cool temperate rain forest), Emu ground (dry sclerophyll forest) and Turquoise Bluff (dry sclerophyll forest) and the soil depth studied was 5 cm. The results of their study are summarised in Table 3. 3.

Table 3. 3 Summary of soil nutrient levels and associated parameters measured by Adams *et al.* (1989) in Nothern Tasmania forests.

Site	Total av. N ($\mu g\ g^{-1}$)	Labile N ($\mu g\ g^{-1}$)	Inorganic P ($\mu g\ g^{-1}$)
Saddleback forest	31.7	57.3	19.7
Emu ground forest	5.1	17.7	2.5
Turquoise Bluff forest	4.7	20.0	1.8

Adams *et al.* (1994) studied nitrogen (N) and phosphorus (P) availability in Heathlands at Wilsons Promontory, Victoria. The values they obtained are summarised as follows (Table 3.4).

Table 3. 4. Summary of soil nutrient levels and associated parameters measured by Adams *et al.* (1994) at Heathlands in Wilsons Promontory, Victoria.

Available N	20.00 ($\mu g\ g^{-1}$)
Available P	1.81 ($\mu g\ g^{-1}$)
Total N	0.17 (%)
Total C	2.74 (%)

Joffre (1990) found that total available nitrogen in grasslands in south western Spain varied from 1.7 to 21.7 ppm (ug g^{-1}) in different seasons. Higher levels of soil nitrogen were observed during dry periods. The climate of the study area was subhumid Mediterranean type with temperate winters. The average mean annual rain fall was 648 mm.

Seastedt *et al.* (1991) studied nitrogen concentrations (NO_3^- - N) in the soil water in tall grass prairie in the USA. They found that soil water nitrate nitrogen concentrations varied from 20 μg L^{-1} to 40 μg L^{-1}.

3.1.2 Mineralization studies

Joffre (1990) found that the rate of nitrogen mineralization changes with the season and the type of plants (perennial or annual) in grasslands. The cumulative mineral nitrogen produced *in- situ* during the growing season (Oct. 1985 - June 1986) in soil under annual grass was 27 μg g^{-1} and in soil under perennial grass was 37 μg g^{-1}. The cumulative potential mineralization in vitro was 173 μg g^{-1} and 101 μg g^{-1} for soil supporting perennials and annuals respectively. The average mineral nitrogen produced in- situ was 0.19 μg g^{-1} day^{-1} and 0.14 μg g^{-1} day^{-1} for soil supporting perennial and annuals respectively. The average mineral nitrogen produced *in-vitro* was 0.88 μg g^{-1} day^{-1} and 0.52 μg g^{-1} day^{-1} for soil under perennials and annual respectively. All results were expressed on a dry weight basis

Adams *et al.* (1989) found that there was no marked seasonal variation in the rates of nitrogen mineralization in forest soil in Tasmania because in some months microbial immobilisation was dominant. Mean rate of nitrogen mineralization varied from 0.09 μg g^{-1} day^{-1} (Emu ground) to 0.28 μg g^{-1} day^{-1} (Retreat forest). The results were expressed on a dry weight basis.

3.2 Methods

3.2.1 Soil nutrients and other physico - chemical properties

The study reported in this section was performed at Derrimut and Laverton grassland reserves. Soil cores were taken at 2 cm - 5 cm soil depth and analysed for nutrients (N, P and K) and other physico-chemical properties according the methods described in chapter two, section 2.3.

3.2.2 *In - vitro* mineralization

Anaerobic (waterlogged) N mineralization *in-vitro* was estimated using the method described by Keeny (1982). A total of 40 *in-vitro* mineralization tubes were prepared according to the method outlined below. At 0, 7, 14, 21 and 28 days eight of these tubes were taken randomly from the set and analysed for extractable NH_4^+- N. To prepare each tube about 5.00g of fresh soil was accurately weighed in a plastic vial for each sample. Sufficient de-ionised water was added to cover the soil sample completely. Each vial was fitted with an air tight "suba-seal" stopper and the content was shaken well so as to produce a slurry. N_2 gas was passed through the soil slurry to create fully anaerobic conditions. Then the vials were placed in an air circulated incubator at 40°C. At the appropriate time extractable NH_4^+- N was analysed by the method described in chapter two.

The rate of mineralization was calculated as follows :

$$\text{rate of mineralization of N} = \frac{\text{final } NH_4^+\text{- N } (\mu g\ g^{-1}) \text{ after 21 days - initial } NH_4^+\text{- N } (\mu g\ g^{-1})}{\text{number of days (21 days)}}$$

The total mineralizable nitrogen content of the soil was taken to be indicated by the total amount of NH_4^+- N produced after 28 days. In all mineralization experiments the amount of NH_4^+ - N present after 28 days was not significantly different from that produced after 21 days, suggesting that mineralization had been substantially completed (see Fig. 3.1 for example).

3.3 Results

Soil pH at Derrimut and Laverton grassland reserves is shown in Table 3. 5.

Table 3. 5 Soil pH at Derrimut and Laverton grassland reserves in autumn 1996 and autumn 1998.

season	Derrimut grassland reserve	Laverton grassland reserve
Autumn (June 1996)	6.15 (0.07)	6.00 (0.07)
Autumn (June 1998)	6.3 (0.1)	6. 12 (0.05)

Mean values of pH in autumn (1996) and autumn (1998) at Derrimut and Laverton grassland reserves. Standard errors are in brackets.

During the study period the levels of total extractable nitrogen (N), extractable phosphorus (P) and extractable potassium (K) in the surface soil (2 cm - 5 cm soil depth) at Derrimut and Laverton grassland reserves are shown in Table 3.6.

Total extractable nitrogen (N) ranged between 2.16 (0.56) - 5.96 (0.58) $\mu g\ g^{-1}$ dry wt. soil at Laverton grassland reserve and 1.33 (0.14) - 4.9 (0.6) $\mu g\ g^{-1}$ dry wt. soil at Derrimut grassland reserve (Table 3.6).

The extractable phosphorus (P) in soil at Laverton grassland reserve varied from 1.44 (0.31) to 5.38 (0.34) $\mu g\ g^{-1}$ dry wt. of soil and at Derrimut grassland reserve changed from 1.19 (0.09) to 4.91 (0.23) $\mu g\ g^{-1}$ dry wt. of soil (Table 3.6).

The extractable potassium (K) in the soil ranged between 272 (23) - 328 (20) μg g^{-1} dry wt. of soil and 221 (14) - 304 (23) μg g^{-1} during study period in soil at Derrimut and Laverton grassland reserves respectively (Table 3.6).

Table 3. 6 Extractable soil nutrients (Nitrogen (N), Phosphorus (P) and Potassium (K)) at Derrimut and Laverton grassland reserves (minimum and maximum levels measured during study period).

site	total extractable N (μg g^{-1} dry wt.)	extractable P (μg g^{-1} dry wt.)	extractable K (μg g^{-1} dry wt.)
Derrimut grassland reserve	1.33 (0.14) - 4.9 (0.6)	1.19 (0.09) - 4.91 (0.23)	272 (23) - 328 (20)
Laverton grassland reserve	2.16 (0.56) - 5.96 (0.58)	1.44 (0.31) - 5.38 (0.34)	221 (14) - 304 (23)

Mean values of extractable nutrients (N, P and K) are expressed in μg g^{-1} (dry weight of soil). Standard errors are in brackets.

The levels of total nutrients (N, P and K) in the soil measured at same season in two years (autumn 96 and autumn 98) at Derrimut and Laverton grassland reserves are described in Table 3.7.

Table 3. 7. Levels of total soil nutrients (N, P & K) measured during study period (1996 - 1998) at Derrimut and Laverton grassland reserves.

site	total N (%)	total P (%)	total K (%)
Derrimut grassland reserve	0.16 - 0.21	0.016 - 0.029	0.30 - 0.40
Laverton grassland reserve	0.15 - 0.19	0.015 - 0.024	0.25 - 0.34

Mean values of total levels of N, P and K. Standard errors are in brackets (n = 8).

Table 3. 8 : Organic carbon (C) contents of soil at Derrimut and Laverton grassland reserves (minimum and maximum levels measured during study period)

site	organic carbon (%)
Derrimut grassland reserve	4.05 (0.28) - 4.69 (0.31)
Laverton grassland reserve	4.02 (0.15) - 4.86 (0.31)

Mean values of organic carbon are expressed in % (dry weight of soil).
Standard errors in brackets (n = 8).

Table 3. 9 Moisture content of soil samples at Derrimut and Laverton grassland reserves (minimum and maximum levels measured during study period)

site	moisture content (%)
Derrimut grassland reserve	5.40 (0.33) - 29.78 (0.59)
Laverton grassland reserve	3.78 (0.25) - 23.11 (0.45)

Mean values of moisture contents. Standard errors are in brackets (n = 8).

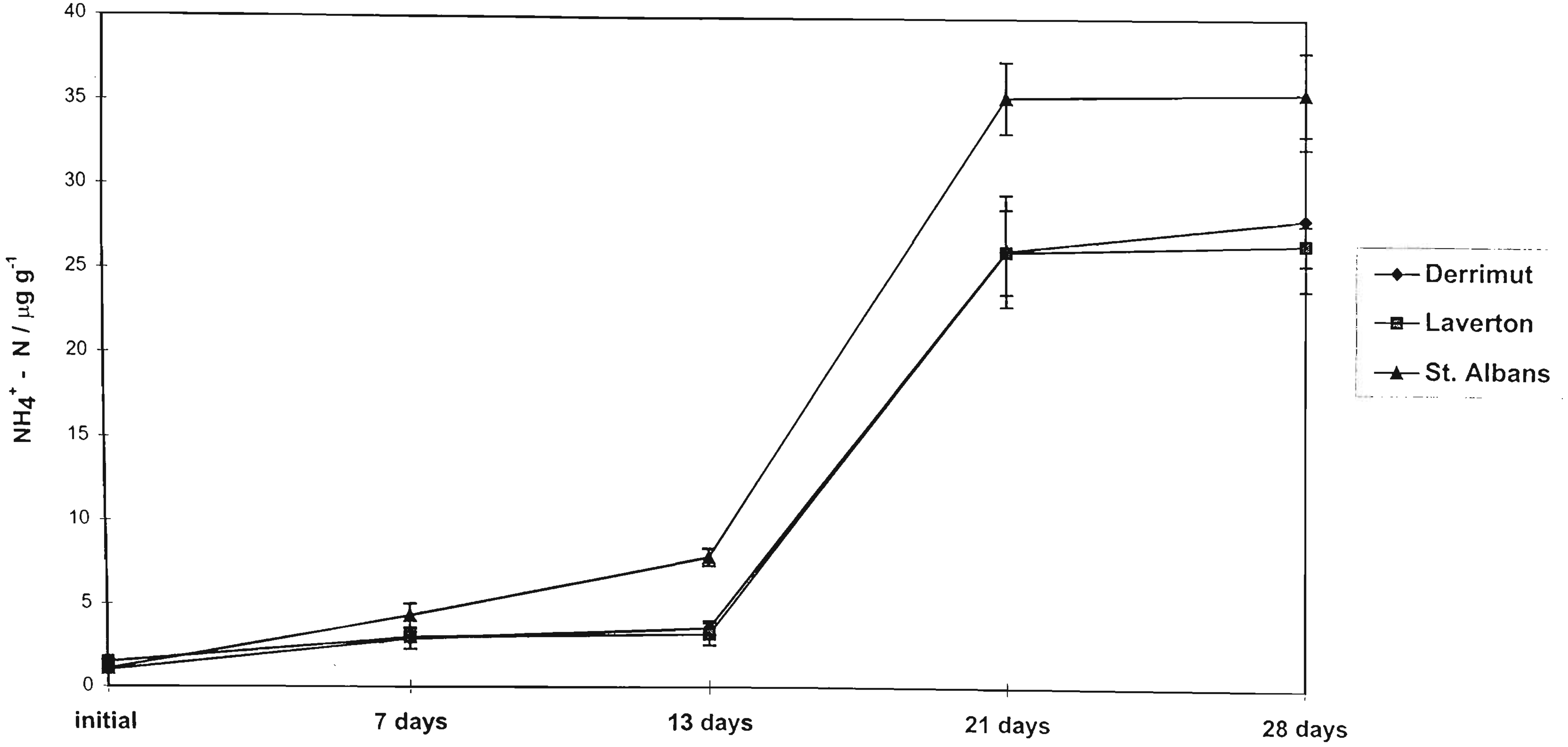

Fig. 3.1. Time course mineralization of nitrogen (N) at Derrimut, Laverton and VUT, St. Albans grassland reserves. Bars indicate standard error (n = 8).

The rates of *in-vitro* mineralization of nitrogen (N) were not significantly different between the two experimental sites, Derrimut and Laverton grassland reserves. By comparison, the rate of mineralization of nitrogen (N) at St. Albans grassland was significantly higher than other three sites (Table. 3.10).

Table 3. 10 : Rates of *in-vitro* mineralisation of nitrogen (N) and total mineralizable nitrogen (N) at St. Albans, Derrimut and Laverton grassland reserves.

site	rate (μg g^{-1} day^{-1})	total (μg g^{-1} dry wt.)
St.Albans grassland reserve	1.64 (0.10)	35.61 (2.45)
Derrimut grassland reserve	1.20 (0.15)	28.17 (4.21)
Laverton grassland reserve	1.18 (0.11)	26.67 (1.18)

Rates of mineralisation of N expressed as μg g^{-1} (dry weight of soil) day^{-1}

Standard errors are in brackets (n = 8).

Mean value of total mineralizable N is expressed as μg g^{-1} (dry weight of soil) produced at each site after 28 days. Standard errors are in brackets (n = 8).

3.4 Discussion

A summary of the comparative results of soil nutrient levels between lowland native grasslands and other ecosystems is outlined in Table 3. 11. Overall the available soil nutrients nitrogen (N), phosphorus (P) and potassium (K) in western (basalt) plains grassland remnant sites were comparatively lower than forest ecosystems.

Table 3. 11 Comparative soil nutrient levels between lowland native grasslands and other ecosystems.

ecosystem	Available N ($\mu g\ g^{-1}$)	Available P ($\mu g\ g^{-1}$)	Available K ($\mu g\ g^{-1}$)	Total N (%)	Total P (%)	Total K (%)	Total C (%)	Mineralizabl N $\mu g\ g^{-1}$ (28 days)
Primary rain forest (Queensland)	45 - 61	-	487 - 815	0.58 - 0.81	0.198 - 0.314	-	6.71 - 11.08	
Cool temperate rain forest (Tasmania)	31.7	19.7	-	-	-	-	-	
Dry sclerophyll forests (Tasmania)	4.7 - 5.1	1.8 - 2.5	-	-	-	-	-	
Heathlands (Victoria)	20.00	1.81	-	0.17	-	-	2.74	
Derived grasslands (Queensland)	38 - 45	-	340 - 573	0.28 - 0.56	0.153 - 0.48	-	5.13 - 8.33	
Snow-tussock grassland (New Zealand)	21	11	-	0.4	-	-	8.1	
Subhumid Mediterranean grassland (Spain)	1.7 - 21.7	-	-	-	-	-	-	37 (perennial) 27 (annual)
Lowland native grasslands (Australia)								
- Derrimut	1.33 - 4.9	1.19 - 4.91	272 - 328	0.16 - 0.21	0.016 - 0.029	0.30 - 0.40	4.05 - 4.69	28.17
- Laverton	2.16 - 5.96	1.44 - 5.38	221 - 304	0.15 - 0.19	0.015 - 0.024	0.25 - 0.34	4.02 - 4.86	26.67
Arid woodland (USA)	-	-	-	-	-	-	-	22.6
Pinus taeda forest (USA)	-	-	-	-	-	-	-	34.8
Nothern hard wood forest (USA)	-	-	-	-	-	-	-	37.7

3.4.1 Available soil nutrients

3.4.1.1 Total extractable nitrogen (N)

Total extractable ammonium nitrogen (NH_4^+ - N) plus nitrate nitrogen (NO_3^- - N) were taken to be an estimate of total extractable nitrogen. During the study period total extractable nitrogen (N) in the surface soil (2 cm - 5 cm soil depth) ranged between 2.16 (0.56) - 5.96 (0.58) $\mu g\ g^{-1}$ dry wt. soil at Laverton grassland reserve (standard errors in brackets) and 1.33 (0.14) - 4.9 (0.6) $\mu g\ g^{-1}$ dry wt. soil at Derrimut grassland reserve and the levels were affected by the season of sampling (see chapter eight for details). The available nitrogen in temperate lowland native grasslands was comparatively lower than found in other high productive ecosystems (e.g. primary rain forests and derived grasslands in Queensland, cool temperate rain forests (South eastern Australia) but overlapped the range of levels found in dry sclerophyll forests in Tasmania (Table 3. 11). Two surprisingly high levels of extractable nitrogen (referred to in the publications as available nitrogen) have been reported for heathland at Wilsons Promontory in Victoria and snow - tussock grasslands in New Zealand (Table 3. 11). These results demonstrate that available nitrogen levels in grasslands and Heathlands are not lower *per se* than for other natural communities. Total available nitrogen fluctuates in the soil because of changes in rates of nitrogen fixation, leaching, nutrient uptake by plants and nutrient immobilisation by soil micro-organisms during different seasons. It is likely that a combination of these factors are at work to produce the low levels of available nitrogen measured in lowland grasslands. Further evidence for this will be presented in later chapters of this thesis.

The pattern of results obtained in the study reported in this thesis are broadly similar to those of other studies of available nitrogen in comparable temperate grasslands, suggesting that the methods for estimating total available nitrogen are reliable.

4.1.2 Extractable phosphorus (P)

The extractable phosphorus (P) in study sites varied dramatically during study period. The extractable phosphorus (P) in soil at Laverton grassland reserve varied from 1.44 (0.31) to 5.38 (0.34) $\mu g\ g^{-1}$ dry wt. of soil (standard errors in brackets) and at Derrimut grassland reserve changed from 1.19 (0.09) to 4.91 (0.23) $\mu g\ g^{-1}$ dry wt. of soil. These levels were in a similar range to the available phosphorus (P) in forest soils (Adams *et al.* 1989) in Tasmania and Heathlands at Wilsons Promontory, but lower than high productivity rainforests (Table 3. 11). There was no marked seasonal variation, but available phosphorus (P) increased substantially in 1998. This change was similar at both sites, Derrimut and Laverton grassland reserves (see chapter eight). The overall increase in available phosphorus (P) might be a result of mineralization of dead plants, due to longer dry period in 1997 and 1998 years. If this were so mineralized phosphorus (P) may have accumulated, resulting higher available phosphorus (P) content in the soil.

3.4.1.3 Extractable potassium (K)

The extractable potassium (K) in the soil ranged between 272 (23) - 328 (20) $\mu g\ g^{-1}$ dry wt. of soil (standard errors in brackets) and 221 (14) - 304 (23) $\mu g\ g^{-1}$ during study period in soil at Derrimut and Laverton grassland reserves respectively. The extractable potassium (K) was not significantly different between seasons or between the two sites, Derrimut and Laverton grassland reserves (see chapter eight). The available potassium (K) in these two grassland reserves were marginally lower than the available potassium (K) in derived grasslands (343 $\mu g\ g^{-1}$- 573 $\mu g\ g^{-1}$), and primary rain forests (487 $\mu g\ g^{-1}$ - 815 $\mu g\ g^{-1}$).

3.4.2 Total nutrient levels

The "total level" of each nutrient N, P and K of soils, provides a measure of the amount of underlying substrate undergoing decomposition. This includes the sum of available and unavailable forms of each nutrient (see chapter one). The levels of total nutrients in the soil measured at same season in two years (autumn 96 and autumn 98) were not same. The levels of total nitrogen (N) and total phosphorus (P) were slightly lower in 1998 than in 1996. The ranges are given in Table 3.11. This difference was observed at both sites Derrimut and Laverton grassland reserves. The level of total potassium (K) was not considerably changed. The total nitrogen (N), was about one third lower in Derrimut and Laverton grassland reserves than in derived grasslands, secondary forests and primary rain forests (Table 3.11). By comparison, total phosphorus (P) at Derrimut and Laverton grassland reserves was in the order of one tenth of the levels in high productivity forests and derived grasslands. This suggests that, in the long term, phosphorus might be acting more than nitrogen to limit high productivity growth in lowland native grasslands.

3.4.3 Mineralization

3.4.3.1 Rate of *in-vitro* mineralization

This study gives an index of nitrogen that is potentially available in the soil and the potential rate at which the nitrogen becomes available. The rates of *in-vitro* mineralization of nitrogen (N) were not significantly different between the two experimental sites, Derrimut and Laverton grassland reserves (Table 3.10). By comparison, the rate of mineralization of nitrogen (N) at St. Albans grassland was significantly higher than other three sites (Table. 3.10). The rate of mineralization of nitrogen (N) at St. Albans grassland was 1.64 (0.10) $\mu g\ g^{-1}$ (dry weight) day $^{-1}$. The reason for this might be because of the season in which the measurements were made. Soil samples for the pilot study to develop the method for measuring *in-vitro* mineralization were collected at St. Albans grassland in summer (March 1996), whereas samples were collected for the main study from the other sites in autumn (May 1996). Generally in summer more easily mineralizable organic matter is produced by plants and micro-organisms (Birch 1960; Kieft *et al.* 1987) which results in increased rates of mineralization than in other seasons. According to Blinky and Hart (1989) the net production of inorganic nitrogen (N) during mineralization is not a linear function of the length of incubation. The variation in nitrogen (N) production from a linear rate during mineralization can be attributed to the balance between mineralization (i.e. production) and immobilisation (i.e. uptake) by microbial decomposers and overall to the dynamics of microbial population growth. The ammonium ion (NH_4^+) flush often observed during anaerobic incubation has been thought to come from the decomposition of aerobic micro-organisms by anaerobic bacteria (Adams & Attwill 1986; Paul *et al.* 1986; Myrold 1987).

For the *in-vitro* mineralization measurements on soil from Derrimut and Laverton grassland reserves until 13 days the rate of nitrogen (N) mineralization was relatively slow (Fig. 3.1). This might be because of immobilisation of the nitrogen produced in early stages of decomposition by micro-organisms. After 13 days, as organic carbon (C) levels became limiting the high microbial population would then begin to die, releasing ammonium ion (NH_4^+) and adding to a faster rate of nitrogen (N) production. The results were similar in soil from the three sites. The mineralizable nitrogen reached a maximum between 25 to 30 days for soil from each of the sites tested (Fig. 3.1). The maximum recorded mineralization of nitrogen in Nothern hardwood forest in USA was 1.3 $\mu g\ g^{-1}$(dry weight) day^{-1} (Myrold and Tiedje 1986). Compared to this value, a similar potential rate of mineralization of nitrogen occur in lowland grasslands. Total mineralizable N for the two lowland grassland sites (Derrimut and Laverton) were similar to values for open forest and woodland ecosystems (Table 3. 11). This strongly suggests that there is high amount of potentially available nitrogen locked up in the vegetation in the soil of the native grasslands.

It is interestingly to note that the rate of *in-vitro* mineralization of nitrogen was very high (about 16 times greater) compared to the rate of *in-situ* mineralisation of nitrogen (see chapter four), suggesting that the rate of decomposition in the field is limited by factors other than availability of micro-organisms or mineralizeable nitrogen.

3.4.3.2 Levels of potentially mineralizeable nitrogen (N)

The very low levels of available N in lowland grassland communities (Table 3. 11) suggests that the plant species present are able to extract available nitrogen from the soil with a high degree of efficiency, and that competition below ground for nitrogen may be an important factor in determining which plant species are able to grow on site. The high levels of potentially mineralizeable nitrogen, combined with the rapid potential rates of mineralization, suggest that the very low levels of nitrogen in intact lowland grassland remnants might increase dramatically if the conditions for mineralization were present, for example after soil disturbance and death of plants. This possibility was the subject of soil disturbance studies reported in chapter 4.

Chapter 4

The effects of physical disturbance on rates of mineralization and available levels of nitrogen (N), phosphorus (P) and potassium (K) in grassland soils.

4.1 Introduction

4.1.1 Disturbance

Disturbance is a major factor affecting the diversity of plant species in natural communities (Connell 1978; Huston 1979). Often, large number of short lived species (annuals and biennials) invade freshly disturbed areas (Sprugal 1985). Disturbance can cause either an increase in plant diversity, or a decrease in diversity, depending on the particular competitive responses of the species available in the seedbank, and the scale at which the disturbance takes place (Muir and Carr 1994). Disturbances can be classified into natural and artificial disturbances. Examples of natural disturbance are fire (lightning), grazing, drought, landslides, trampling by animals, mound building and digging of burrows. Artificial or human caused disturbances include fire, altered fire regimes, feral grazing, mowing and digging.

According to the definition by Picket and White (1989) , " A disturbance is any relatively discrete event in time that disrupts ecosystem, community or population structure and changes resources substrate availability or the physical environment". Disturbance can be short term and small scale or long term and large scale according to the type of disturbance. Disturbances often create patches or gaps in the vegetation canopy and changed conditions affecting plant growth below ground. The patchy environments in grasslands help to maintain species composition and biological diversity (Lavine & Paire 1974; Platt 1975; Giesel 1976; Wiens 1976).

Physical disturbance of soil temporarily creates openings or gaps in the vegetation canopy and in turn increases availability of both light and temperature at ground level. Gutterman (1985) and Brady (1984) each make the point that light and temperature affect seed germination and algal growth in disturbed gaps in forest ecosystems. McGee (1976) found that large scale clear cutting of forests produced higher maximum and lower minimum temperatures than closed forests. Miller (1969a) and Skeen (1976) have also pointed out that higher light intensity will be received at ground level in disturbance gaps.

A number of studies have shown that soil disturbance increases available nutrients in the soil. Physical disturbance disrupt nutrient cycles by interrupting plant uptake of nutrients and this in turn leads to accumulation of nutrient levels (especially nitrogen) in the soil (Sprent 1987). Matson and Vitousek (1981) have pointed out that physical soil disturbance leads to increased mineralization of nitrogen (N), phosphorus (P) and sulfur (S) and consequently to increases of their levels in the soil. Swift *et al.* (1975) have found that removing plants by disturbance in forest ecosystems leads to greater water flux through the soil, thus increasing nutrient losses by leaching and denitrification. Where large amounts of nitrate ions (NO_3^-) are lost from soil by leaching, calcium (Ca^{2+}), magnesium (Mg^{2+}) and potassium (K^+) losses also increase as cations are displaced from soil exchange sites to maintain electrochemical neutrality (Nye & Grrenland 1960; Borman & Likens 1979).

4.1.2 Organic matter decomposition and mineralization

Soil organic matter plays a major role in terrestrial ecosystems development and functioning by its special physical and chemical properties. Soil organic matter is a major source of nutrients for plants, contributing around 95 % of soil nitrogen (N) 40 % of soil phosphorus (P) and 90 % of soil sulfur (S) (Jeffrey *et al.* 1993). Therefore, the decomposition and mineralization of organic matter supplies the major portion of macro-nutrients for the plant growth.

Soil organic matter is a complex mixture of living, dead and decomposing materials. Most of the soil organic matter is derived from plant materials, but some is formed from soil fauna including micro-organisms (Jeffrey *et al.* 1993).

Disturbed soil is exposed to sunlight, is highly aerated, has high water filtration and contains higher biomass of dead plant materials than undisturbed soil, because soil disturbances invariably leads to some plant death. These factors are favourable for decomposing micro-organisms. Dormaar (1992) found that solar radiation increases soil temperature which is favourable to growth of micro-organisms. Soil micro-organisms can also be affected by availability of water.

Grassland soils usually have low levels of available nutrients and high levels of organic matter and organically bound nitrogen (N) and phosphorus (P). The accumulated organic matter undergoes rapid mineralization as soon as the sward is ploughed (Theron & Haylett 1953; Harmsen & Schreven 1955; Sprent 1987).

In grasslands the major input of organic substances into the soil is through decomposition of the root biomass (Dormaar 1992). In forests there is typically more visible and higher organic content of litter at the soil surface, whereas in grasslands surface litter is of secondary importance and as much as 90% of plant biomass occurs underground although root biomass is usually around 65 % of total biomass. Kochy and Wilson (1996) found that the N content of grass litter was higher than forest (aspen) litter and the rate of decomposition of grass litter was 3 times greater than forest litter. Joffre (1990) found higher nitrogen content in perennial grasses than in annual grasses.

The overall purpose of the studies reported in this chapter were to investigate the rates and extent of nutrient release due to mineralization in the disturbed soil of a lowland plains grassland remnant.

4.2 Methods

4.2.1 Soil disturbance - design of experimental plots

This study was performed at Derrimut grassland reserve between early spring to early summer 1997. Circular plots of 1m diameter were used for the experiments to minimise corner effects. All plots were mown initially to 4 cm plant height from the ground and overburden removed by raking. Plots were randomized through out for both controls and dug (disturbed) treatments, each treatment consisting of 8 replicates. Each plot chosen for dug treatment was manually dug to a depth of 15cm in two stages. The first layer removed was 7cm deep from the ground level and second layer was at 7cm- 15cm. Soil layers were kept separate and laid out on plastic sheeting. Each layer was manually homogenised so as to produce small particle sizes (less than 1cm) and returned to the hole in their original order. Plots were left for 14 days to allow the soil to stabilise before carrying out any analysis.

4.2.2 *In-situ* Mineralization (field incubation) in dug and control plots.

In-situ mineralization was determined using the method outlined in Raisen *et al.* (1987). PVC tubes (4 cm diameter x 11 cm long) with the lower edge sharpened were driven 8 cm into the ground. Small holes were drilled into the exposed sides (ie. above ground level) to allow for gas exchange with the air. Tubes were covered with lids to prevent possible entry of rain water and leaching losses of available nitrogen and phosphorus. *In-situ* mineralization tubes were placed in both experimental dug plots and control undug plots.

4.2.2.1 Mineralization of nitrogen (N)

Total extractable N (NH_4^+ - N plus NO_3^- - N) in soil samples (2 cm - 5 cm soil depth) taken next to the test site was analysed at the time that the PVC tubes were initially placed in the ground. Thereafter, total extractable nitrogen (N) inside the tubes (2 cm - 5 cm soil depth) was analysed at 14, 34 and 70 days after digging (i.e. 0, 20 and 56 days after the PVC tubes were inserted). Total extractable nitrogen (NO_3^- + NH_4^+) was analysed by the methods described in chapter 2. The rate of mineralization was calculated as follows.

$$\text{The rate of mineralization} = \frac{\text{final total extractable N - initial total extractable N}}{\text{number of days}}$$

4.2.2.2 Mineralization of phosphorus

The extractable phosphorus (P) in soil samples (2 cm - 5 cm soil depth) taken next to the test site was analysed at a time the PVC tubes were initially placed on the ground. Thereafter, extractable phosphorus (P) inside the tubes (2 cm - 5 cm soil depth) was analysed at 14, 34 and 70 days after digging (i.e. 0, 20 and 56 days after the PVC tubes were inserted). The extractable phosphorus (P) was analysed by the methods described in chapter 2. The rate of mineralization was calculated as follows.

$$\text{The rate of mineralization} = \frac{\text{final extractable P - initial extractable P}}{\text{number of days}}$$

4.2.3 Available soil nutrients in dug and control plots

Extractable soil nutrients (N, P and K) in 2 cm - 5 cm soil depth were analysed in soil samples taken from disturbed and undisturbed plots at 14 days, 34 days and 70 days after digging. The methods of analysis are described in chapter 2, section 2.3.

4.3 Results

After 70 days there was an approximate ten fold increase in extractable nitrogen (NH_4^+ - N) in the mineralization tubes in undisturbed plots and about twenty fold increase in extractable nitrogen (N) in disturbed plots. Also over 70 days there was an approximate two fold increase in phosphorus (P) in both disturbed and undisturbed plots (Fig. 4.1).

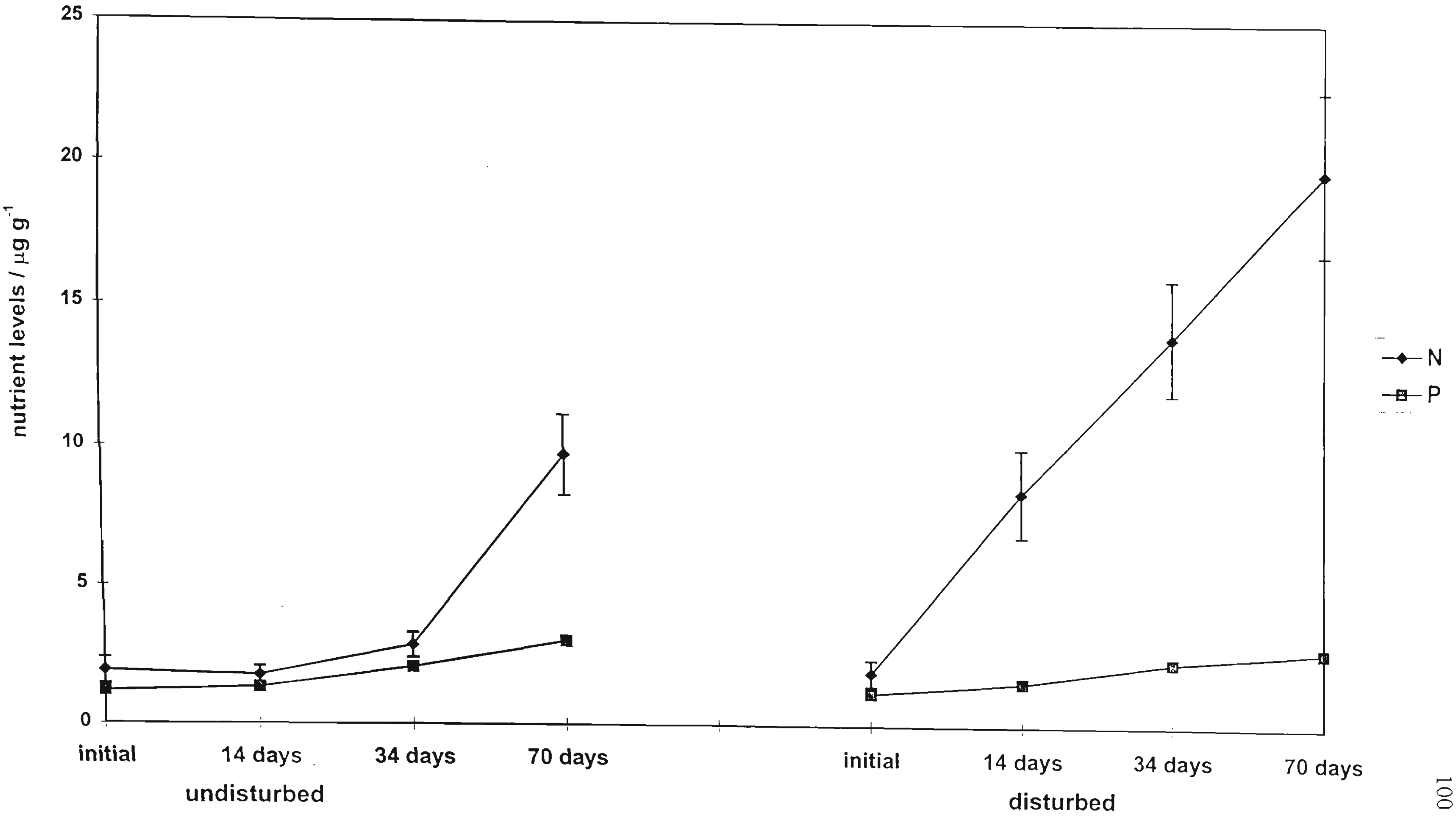

Fig. 4.1. Progressive *in-situ* mineralisation of nitrogen (N) and phosphorus (P) in disturbed and undisturbed grassland plots. Bars indicate standard errors (n = 8).

There was much higher rate of *in-situ* N mineralization in disturbed (dug) plots than in undug (ie. control) plots (Table 4.1). The rate of mineralization of N was more than 2 (>2) times greater in dug plots than in undug plots over 70 days.

Table 4.1. Rates of *in-situ* mineralization (N & P) -disturbed and undisturbed soils.

treatment	N ($\mu g\ g^{-1}\ day^{-1}$)	P ($\mu g\ g^{-1}\ day^{-1}$)
control (undisturbed)	0.11 (0.01)	0.037 (0.0035)
dug (disturbed)	0.25 (0.02)	0.040 (0.0030)

Rates of mineralisation are given in $\mu g\ g^{-1}\ day^{-1}$. Standard errors are in brackets (n = 8).

The extractable phosphorus (P) in undisturbed (undug) and disturbed (dug) at various is described in Table 4.2. The extractable P, at 70 days after digging was significantly higher ($p < 0.01$) in dug plots than undug plots.

Table 4.2. Extractable phosphorus (P) in disturbed and undisturbed plots at various times after digging.

Days	Extractable P (undug)	Extractable P (dug)
initial	1.18 (0.09)	1.18 (0.09)
14	1.33 (0.15)	1.58 (0.12)
34	1.86 (0.16)	2.34 (0.23)
70	1.31 (0.11)	2.32 (0.22)

The mean value of extractable phosphorus (P) is given in $\mu g\ g^{-1}$ dry wt. Standard errors are given in brackets (n = 8).

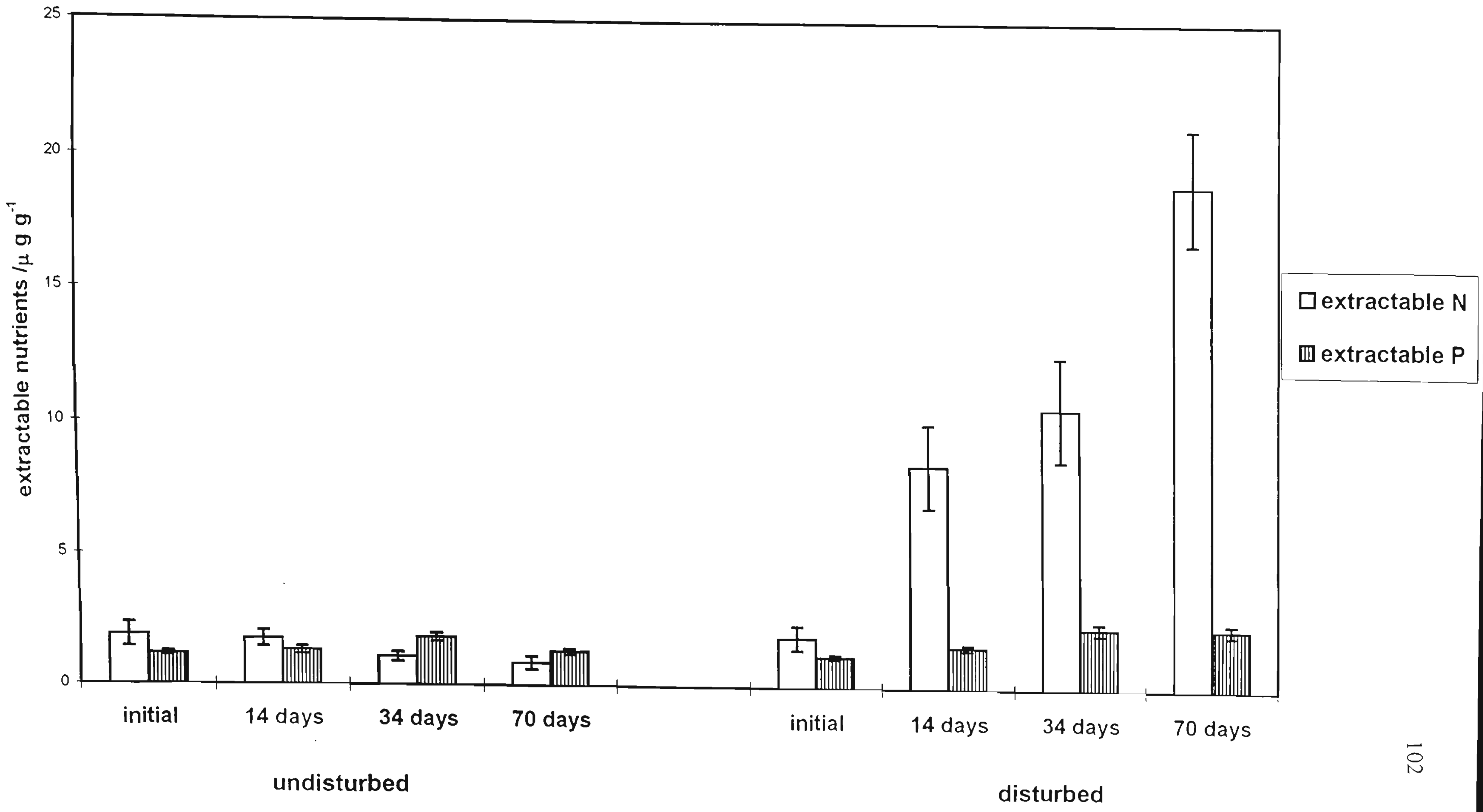

Fig. 4.2. Extractable nitrogen (N) and extractable phosphorus (P) in undisturbed and disturbed plots at various times after digging. Bars indicate standard errors (n = 8)

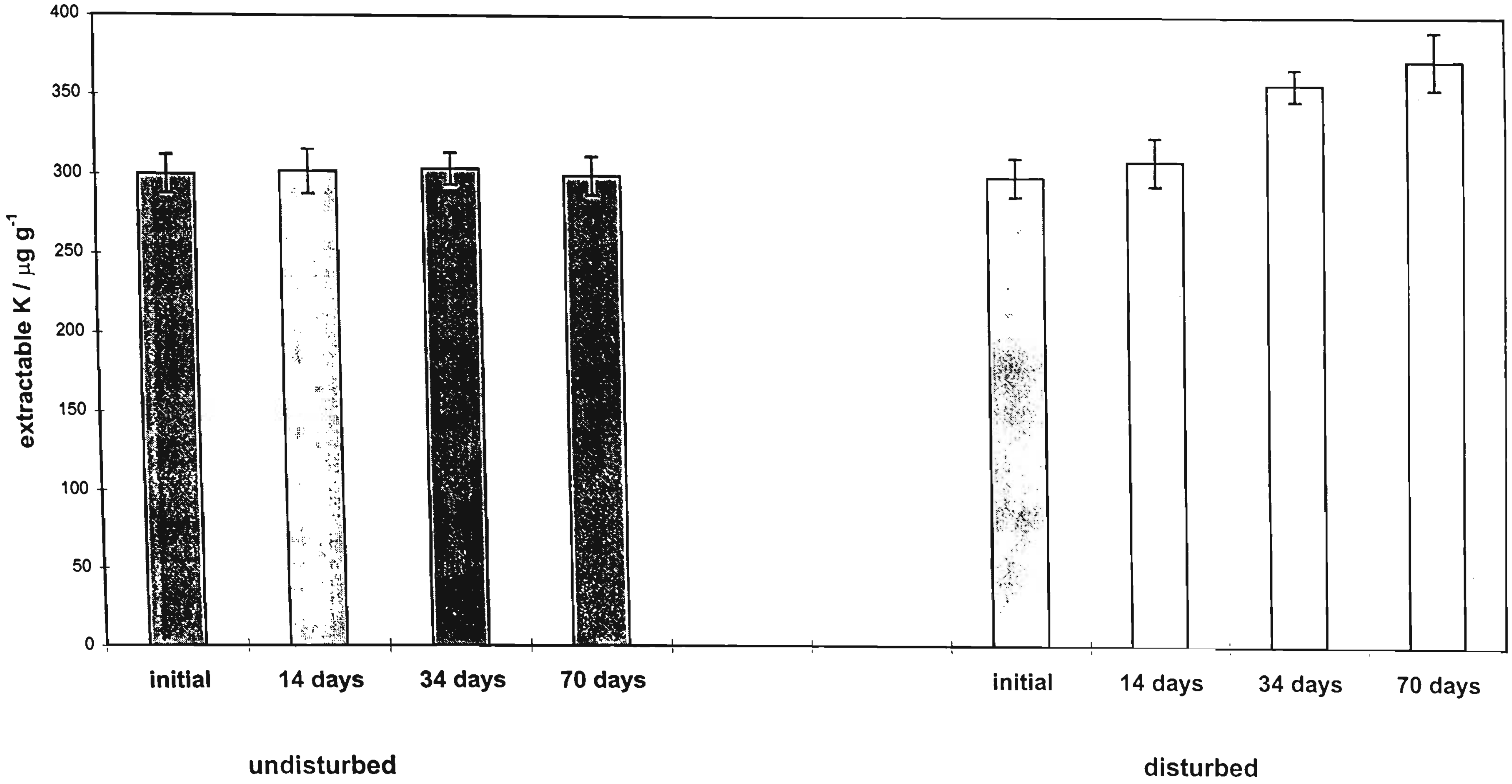

Fig. 4.3. Extractable potassium (K) in disturbed and undisturbed grassland plots at various times after digging. Bars indicate standard errors (n = 8)

Organic carbon is significantly different ($p < 0.01$) between dug and undug plots (Table 4.3).

Table 4.3. Organic carbon content- disturbed and undisturbed plots 70 days after digging at Derrimut lowland grassland remnant.

Treatment	Organic carbon (%)
dug (disturbed)	3.41 (0.15)
undug (undisturbed)	4.21 (0.21)

The mean value (%) of organic carbon of each treatment is given and standard errors are in brackets ($n = 8$). Organic carbon is significantly different ($p < 0.01$) between dug and undug plots.

Soil pH is significantly different between dug and undug treatments ($p < 0.05$) (Table 4.4).

Table 4.4. soil pH levels - disturbed and undisturbed plots

Treatment	pH
dug (disturbed)	6.06 (0.11)
undug (undisturbed)	6.63 (0.15)

The mean value of pH of each treatment is given and standard errors are in brackets ($n = 8$).

pH is significantly different between dug and undug treatments ($p < 0.05$).

Moisture content is significantly different - samples taken during dry weather ($p < 0.01$) at 70 days after digging (Table 4.5 a) and moist weather at 100 days after digging (Table 4.5 b).

Table 4.5 Soil moisture content of disturbed and undisturbed plots at Derrimut grassland reserve.

(a) 70 days after digging

Treatment	Moisture content (%)
dug	4.8 (0.27)
undug	5.8 (0.32)

The mean value (%) of each treatment is given and standard errors are in brackets ($n = 8$). Moisture content is significantly different - samples taken during dry weather ($p < 0.01$).

(b) 100 days after digging

Treatment	Moisture content (%)
disturbed	10.85 (1.03)
undisturbed	7.38 (0.80)

The mean values (%) are shown and standard errors are in brackets ($n = 8$). Moisture content is significantly different ($p < 0.05$) - samples taken during moist weather.

4.4 Discussion

4.4.1 *In-situ* mineralization

The higher extractable nitrogen (N) in disturbed plots would have been largely a result of decomposition of plants killed by placing the tubes over them and cutting off lateral roots. The rate of N production was initially slower in tubes in undisturbed plots, presumably because in these plots slower microbial build up due to less favourable conditions and plants took longer to decompose.

The higher mineralization rate of N in dug plots than in undug plots is expected because dug plots are more open, exposed to the sunlight and more highly aerated than undug plots, all of which would assist the mineralization activity of soil microbes. There would also have been a much greater biomass of dead plant materials available for decomposition early in the experiment in dug plots than in undug plots, resulting from the rapid death of plant root material caused by the digging process. However, large scale release of NH_4^+ - N in undisturbed plots did not begin until after 34 days. Between 34 days and 70 days the rate of mineralization appeared to be similar to that in dug plots. It is possible that rates of mineralization are similar in both treatments, but it takes longer for mineralization to begin in undug plots (ie. for plants to die). This possibility should be investigated further.

The rate of mineralization of P was not significantly different ($p > 0.1$) between dug and undug treatments. Several reasons can be suggested for this. It may be that P is more limiting than N for microbial growth and more of the mineralized P was rapidly used by soil microorganisms in both dug and undug plots during their active growth phase. Microorganisms utilize P freely from the soil. Previous studies have shown that some of phosphate fertilisers added to soil quickly become part of microorganisms body (Brady 1984). Although the microbial biomass in soils is less than that of the higher plants, the P content of microbes per gram of dry weight may be as much as 10 times higher than for plants (Brady 1984). The annual uptake of P by microbes has been found to exceed that of higher plants in forest ecosystems (Brady

1984). In addition to assimilation of P by the soil microbial body, P released from dead plant material during mineralization may have been inorganically bound or fixed with Fe, Ca, Mn or Mg in the soil, or attached to organic matter and may have therefore been less available in the soil.

4.4.2 Available soil nutrients

4.4.2.1 Available nitrogen (N)

This refers to total extractable N (NH_4^+ - N & NO_3^- - N). The total extractable nitrogen in dug plots was significantly higher (Fig. 4.2) than undug plots at various times after the digging treatment. After 34 days, the N content in dug plots was about 10 times greater than in undug plots ($p < 0.001$) and at 70 days after digging the value was 20 times greater than in undug plots ($p < 0.001$). When NH_4^+- N and NO_3^- - N are considered separately in dug plots, up to 34 days NO_3^- - N was much higher than NH_4^+ - N, but at 70 days this balance changed and NH_4^+ - N was about 2 times greater than NO_3^- - N (data not shown). In addition the NO_3^- - N level at 70 days was very similar to the level at 34 days. At 70 days the higher NH_4^+ - N was probably due to a build up of high biomass of microbial decomposers leading to rapid mineralization and the level of NO_3^- - N was almost constant most likely because plants (mainly weeds) growing on the plots were probably absorbing the available NO_3^- - N. Alternatively (or in addition), the conditions allowing nitrifying bacteria to convert NH_4^+ to NO_3^- (eg. moisture, temperature availability) may have changed between 30 days and 70 days, or an increase in release of NH_4^+ may have exceeded the capacity of nitrifying micro-organisms to convert much of the NH_4^+ to NO_3^-.

4.4.2.2 Available phosphorus (P)

The extractable P, at 70 days after digging was significantly higher ($p < 0.01$) in dug (disturbed) than undisturbed plots (Fig. 4.2 & Table 4.2) . Soil pH was significantly lower

($p < 0.01$) in dug plots than undug plots (Table 4.4).

$$Al^{3+}(aq) + H_2PO_4^-(aq) + 2H_2O \Leftrightarrow 2H^+(aq) + Al(OH)_2.H_2PO_4(s)$$

According to the equation describing the binding of phosphate ion ($H_2PO_4^-$) to soil borne aluminium ion (Al^{3+}): at lower pH, there should be higher available P ($H_2PO_4^-$) content in the soil. Lower pH leads to increased availability of P in the soil because unavailable form of P ($Al(OH)_2.H_2PO_4^-$) is converted to available form ($H_2PO_4^-$) at increased concentration of hydrogen ions (i.e. lower pH). These results together suggest that there is likely much higher availability of P in dug plots than in undug plots.

The very low availability of P in the soil in undug plots was probably due to the ongoing uptake of P from the soil by living plants (suggesting that *Themeda triandra* the dominant species in the grassland, is " P hungry") whereas in dug plots the dead plant material was releasing P at rate similar to those seen in the *in-situ* mineralization experiments (Fig. 4.1).

4.4.2.3 Available potassium (K)

The available K, at 34 days after digging was significantly higher ($p < 0.001$) in dug plots than in undug plots (Fig. 4.3). Previously, it has been found that under ground grassland plant materials contain very high levels of mineral ions (Ca, K, Na) compared to above ground materials - up to 20% - 40% (Blanka & Ulenhlova 1989). In the roots of grasses K levels are the second highest metallic element after calcium (Ca). Decomposition of plant roots and other plant materials would be expected to cause major increases in K levels in the soil.

4.4.2.4 Organic carbon

Organic carbon (Table 4.3) was significantly higher ($p < 0.01$) in undug plots than in dug plots at 70 days after digging. The lower organic carbon in dug plots was most likely because of the higher break down rate of organic carbon via microbial respiration compared to undug plots. The lower organic carbon content in the soil of dug plots is consistent with the higher levels of available nitrogen, phosphorus and potassium.

4.4.2.5 Moisture

During moist weather higher moisture content was observed in dug plots than in undug plots and during dry weather dug plots had lower moisture content (Table 4.5). Results were significantly different ($p < 0.01$ in both cases).

The combination of soil moisture, temperature, availability of light, aeration and readily available dead plant materials with high nutrient content (perennial grasses store high nutrient content) in dug plots would have fostered high rates of mineralization (as explained above) and as a result higher available nutrients (N, P & K) in disturbed (dug) plots compared with undug plots.

4.4.3 Disturbance causes high available soil nutrient levels

In summary, the studies reported in this chapter show that physical disturbance of soil under lowland native grassland remnants causes major increases in the levels of available soil nutrients (N, P & K), due to the rapid mineralization of dead plant material produced during disturbance. The gaps created by digging possess two key changed conditions which are conducive for rapid plant growth - removal of above ground vegetation cover and increased availability of nutrients. The effects of these two altered conditions on the potential for weed invasion in soil disturbed areas of lowland native grassland remnants will be explored in chapter five.

Chapter 5

The impact of nutrient addition on the above ground vegetation (natives and weeds) in physically disturbed grasslands.

5.1 Introduction

Competition among plant species for limited resources is a major determinant of species composition and diversity in plant communities, and plays a major role in regeneration and successional dynamics (for example, see: Whittaker 1965; Parrish & Bazzar 1985). In addition to the soil seed bank, resources such as available soil nutrients, soil moisture and light are among the main factors which determine species composition and richness in a given ecosystem. For lowland native grasslands the results reported in chapter 3 demonstrated that physical disturbance (digging) of soil in native grasslands results in much increased levels of available nutrients (nitrogen, phosphorus and potassium). Nutrients, most commonly nitrogen and phosphorus, are among the resources that become limiting for plant growth in natural ecosystems (Vitousek *et al.* 1982; Birk & Vitousek 1986). Application of fertilisers can lead to dramatic shifts in the species composition and diversity of plant communities (Milton 1947; Willis & Yemm 1967; Specht 1963; Ginso *et al.* 1982; Tilman 1984,1986) and there are often strong correlations between soil chemistry and the local distributions of species (Snaydon 1962; Pigott & Taylor 1964; Whittaker & Niering 1975; Christensen & Peet 1984). Bonis *et al.* (1997) found that available phosphorus (P) is more limiting than available nitrogen (N) on gap demanding species in chalk grassland and also that gap demanding species grew more rapidly in lower pH soils than higher pH soils. A number of grassland and shrubland studies have shown that, when resources such as N, P and moisture were abundant in the soil annuals with high growth rates appear in early successional stages and outperform the slower growing perennials (Grim 1979; Leps *et al.* 1982; McGraw & Chapin 1989). However, when resources became limiting, species with lower nutrient requirement usually (perennials) were found to have the advantage (Grim 1979; Leps *et al.* 1982; McGraw & Chapin 1989). McLendon *et al.* (1991) suggest, from studies of nutrient addition to soil disturbed sites in a sagebrush steppe community in north-western Colorado, that dominance of disturbance sites by annuals is a function of seed availability and rapid growth rate, which combine to allow annuals to dominate the supply of available soil nutrients. McLendon *et al.* (1991) found that perennials eventually came to dominate in disturbed plots four years after disturbance, when the soil nitrogen became limiting for plant growth, following an initial rapid growth of annual grasses and forbes.

As part of the studies reported in this chapter, disturbed experimental plots were treated by adding nitrogen (N) or phosphorus (P) nutrients alone, or both nitrogen (N) and phosphorus (P) together, or by introducing low levels of nutrients by adding an organic carbon source (sugar). The above - ground vegetative biomass for each treatment was determined after one growing season, is an attempt to identify which of the nutrients N and P influenced growth, and hence whether either or both of these were limiting.

5.2 Methods

5.2.1 Experimental design

Circular plots of 1 m diameter, including a 20 cm outer buffer zone and inner 80 cm diameter assessment area, were used for this experiment (Fig. 5.1). Plots were dug and set up in early spring (September) of 1997 at Derrimut grassland reserve in an experimental area dominated by *T. triandra*, using the same method reported in chapter 3. Plots were randomized throughout the experimental site and each treatment consisted of eight replicates. Each plot was divided into two halves. One half was used to analyse soil nutrients and the other half to analyse above ground biomass of plants growing on the plots.

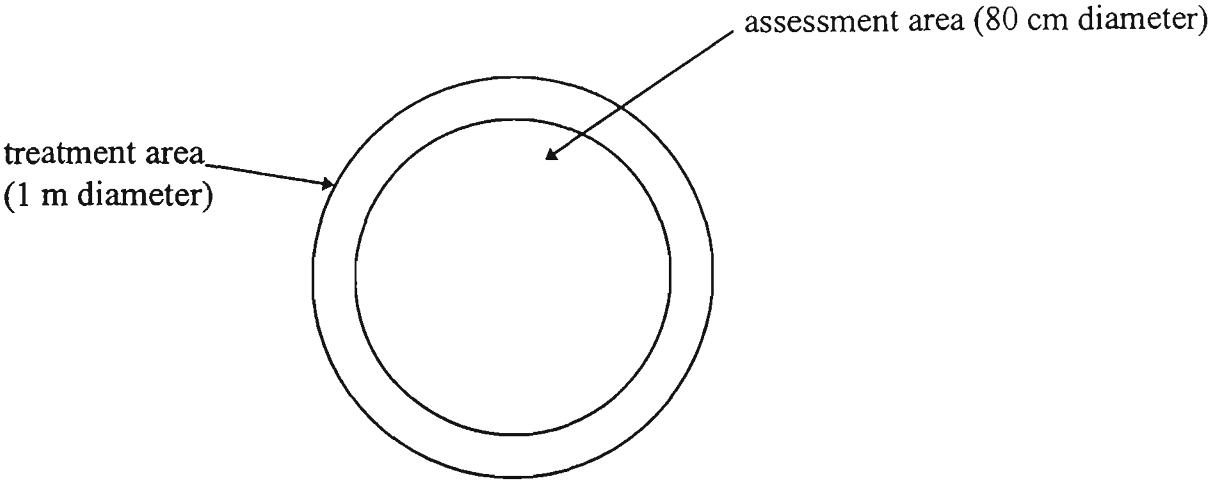

Fig. 5.1. Soil disturbance plot. Outer treatment area is 1 m diameter and inner assessment area is 80 cm diameter.

Treatments of plots were as follows :

- Undisturbed control.
- Disturbed (dug) control.
- Disturbed (dug) and nitrogen (N) added (0.832 g m^{-2} $month^{-1}$ of N as NH_4NO_3).
- Disturbed (dug) and phosphorus (P) added (0.832 g m^{-2} $month^{-1}$ of P as Triple Super Phosphate).
- Disturbed (dug) with nitrogen (N) plus phosphorus (P) added (0.832 g m^{-2} of N as NH_4NO_3 and 0.832 g m^{-2} of P as Triple Super phosphate $month^{-1}$).
- Disturbed (dug) and carbon (C) added (3.32 g m^{-2} $month^{-1}$ of C as Sucrose).

Nutrients were hand broadcast on to experimental plots monthly until end of spring for 3 months.

5.2.2 Soil analysis.

Soil cores were extracted and analysed for extractable N and P according to the procedures in chapter 2, section 2.3 - standard methods for soil nutrient analysis.

5.2.3 Biomass analysis.

After three months of the spring growth (October - December) period the above ground plant materials were clipped to ground level in early summer, separated by species and dried at 70^0C until each sample attained a constant weight.

5.2.4 Statistical analysis

The tests of significance were performed with treatments and following parameters.

1. Treatments and major plant groups (*Themeda*, annual grasses, thistles + other broad leaves).

2. Treatments and extractable nutrients (NH_4^+ - N, NO_3^- - N, PO_4^{3-} - P).

The level of significance is given in brackets with appropriate analysis. Details of statistical analysis are present in appendix 3.

5.3 Results

Table 5.1. Comparison of the average above-ground biomass of major plant groups growing on undisturbed (undug) and disturbed (dug) plots, three months after soil disturbance.

Treatments	*Themeda triandra* grass	Annual grasses	Thistles and other flat weeds	Other exotics	Total
undug	31.79 (4.58) 95.75 %	1.09 (0.87) 3.28 %	0.00 0.00	0.32 (0.27) 0.96 %	33.2 (5.72)
dug	0.23 (0.13) 3.81 %	1.39 (0.33) 23.05 %	4.26 (1.12) 70.65 %	0.15 (0.09) 2.45 %	6.03 (1.67)

The mean biomass of plants is expressed in g m^{-2}. Standard errors are in brackets (n = 8).

Percentages indicate percentage of total biomass in each plant group.

Table 5.2. Major plant species growing in disturbed plots - the diversity of plants in disturbed plots with N or P added was similar.

Thistles and other broad leaved species	Annual grasses
Sonchus oleraceous	*Briza maxima*
Picris echioides	*Briza minor*
Cirsium arvense	*Aira caryophyllea*
Hypochoeris glabra	*Lolium rigidum*
Plantago vairia (native)	

5.3.1 Comparison of plant types and biomass on undisturbed plots and disturbed plots (with no nutrients added)

The analysis of biomass of each of the major plant groups which grew in disturbed and undisturbed plots are summarised in Table 5.1 and Fig. 5.2. Comparison of analysis of plants grown in dug plots and undug plots showed that more than 95% of the biomass of plants in dug plots were weedy species (annual grasses, thistles & other broad leaved species). In contrast, the biomass harvested from plants in undug plots consisted mainly (> 90%) of native *Themeda triandra* grass (Table 5.1). By comparison species in terms of biomass exotic broad leaved species were dominant with exotic annual grasses as subdominant in disturbed plots. Native grass and forbs were almost absent. Broad leaved species in disturbed plots were mainly *Sonchus oleraceus* L. (sow thistle - annual herb), *Picris echioides* L. (Bristly ox-tongue - annual herb), *Cirsium arvense* (L.) Scop. (perennial thistle) and *Hypochoeris glabra*

L. (smooth cat's ear - annual herb). The native *Plantago vairia* R. Br. (sago weed - perennial forb) was also identified in two plots.

Annual grasses in disturbed plots were mainly: *Briza maxima* L., *Briza minor* L., *Aira caryophyllea* L. and *Lolium rigidum* Gaud. (Wimmera Rye Grass). The average biomass of thistles and other broad leaved weedy species was about two times greater than annual grasses in disturbed plots.

5.3.2 Comparison of plant biomass in disturbed plots (with no nutrients added) and disturbed plots with nitrogen (N) or phosphorus (P) added alone.

Overall plant species composition (as measured by biomass) in disturbed plots to which N or P was added alone was similar to that for disturbed plots (no nutrient added) (Fig. 5.2). However the addition of nutrients to disturbed plots affected the biomass of the various plant groups in some instances. There was significantly higher ($p < 0.01$) biomass of annual grasses in P added plots than N added plots (Fig. 5.2). There was also significantly higher ($p < 0.01$) biomass of annual grasses observed in P added plots than in disturbed alone (ie. unfertilised) plots. There was no significant difference ($p > 0.1$) in biomass of thistles plus other broadleaf weeds from disturbed only plots and plots to which either N or P had been added (Fig. 5.2).

Total biomass of plants was not significantly different between dug unfertilised and dug plus N - added alone plots ($p > 0.1$), or between dug unfertilised and dug plus P - added alone treatments ($p > 0.1$).

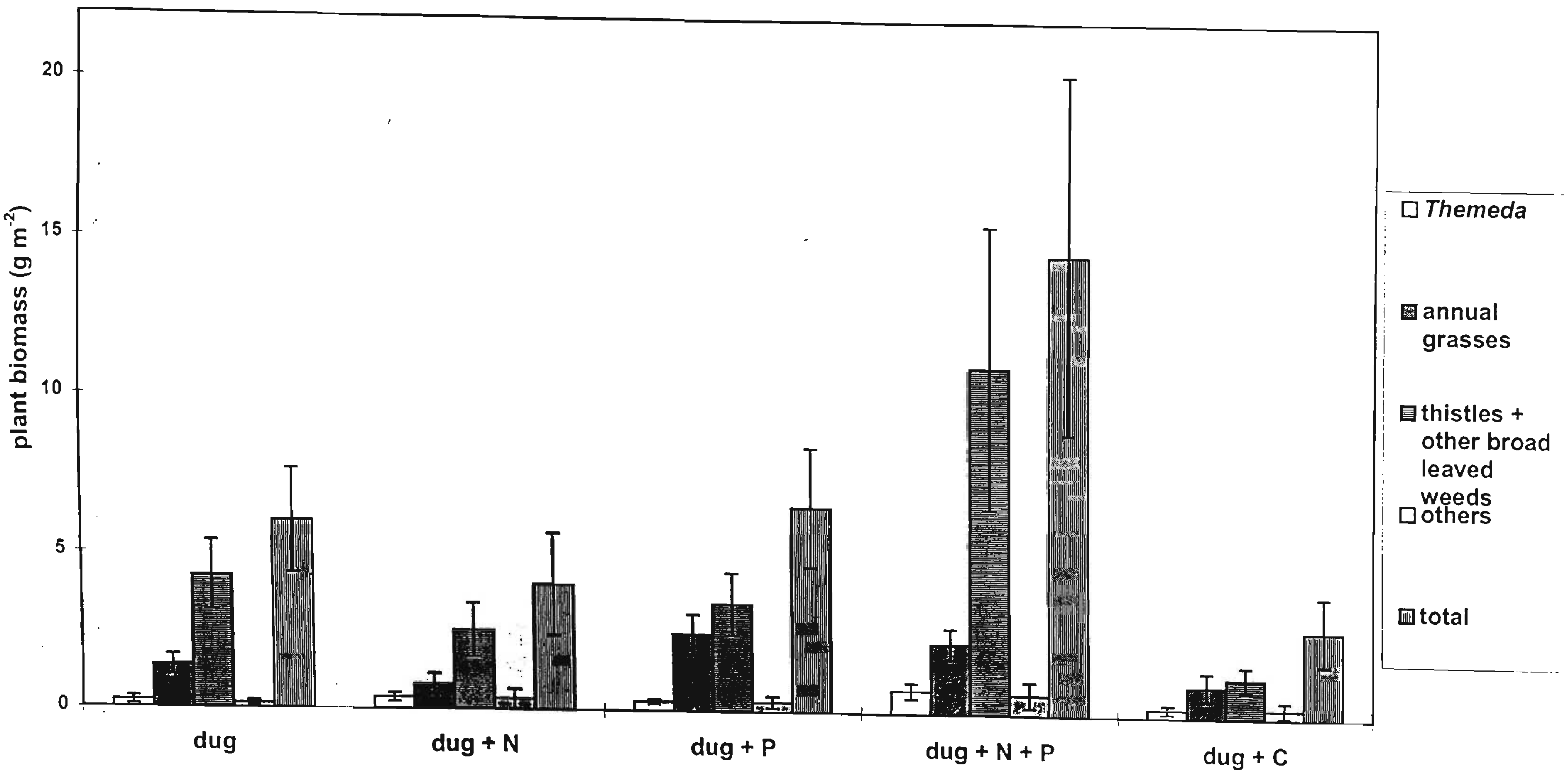

Fig. 5.2. The above ground biomass of disturbed (dug) alone and disturbed (dug) plots with nutrients added, or a carbon source (sucrose) added to induce low N and P levels. Mean values and standard errors (error bars) are shown in Y axis.

5.3.3 Comparison of plant biomass in disturbed plots (with no nutrients added) and disturbed plots with Nitrogen (N) and Phosphorus (P) added together

Total plant biomass in plots to which both N + P were added was very significantly higher ($p < 0.001$ each case) than all other treatments (ie. disturbed only (unfertilised), disturbed + N or disturbed + P) (Fig. 5.2).

However, the overall species diversity was the same on disturbed alone (unfertilised) plots and in disturbed with N + P added plots (Table 5.2, Fig. 5.2). Thistles and other broad leaved weeds were dominant in disturbed plots to which N + P had been added and the biomass of this group of plants was significantly higher than for the same plant groups from each of disturbed alone (unfertilised) plots, disturbed + N added or disturbed + P added plots ($p < 0.001$ in each case).

The total biomass of annual grasses was significantly higher ($p < 0.01$) in disturbed and N + P added plots than disturbed + N alone plots, but annual grass biomass was not significantly different between disturbed and N + P plots and disturbed + P alone ($p > 0.1$ each case).

5.3.4 Comparison of disturbed alone (no nutrients added) plots and disturbed plots with carbon (C) added.

Total above ground plant biomass was significantly lower ($p < 0.005$) in disturbed + C plots than in disturbed alone (unfertilised) plots (Fig. 5.2). The above ground biomass of both annual grasses and broad leaved weeds were also significantly lower ($p < 0.001$) in disturbed + C plots compared to that of disturbed alone (unfertilised) plots.

Estimations of extractable soil nutrients at 70 days after digging are summarised in Table 5.3. Comparison of results show that the extractable N in disturbed plots is about 22 times greater than in undisturbed plots (as explained in chapter four). The results show that adding extra nutrients (N & P) to disturbed plots increased the levels of extractable nutrients in disturbed plots. The levels of extractable N in dug + N plots and in dug + N + P were about three times and four times greater respectively than in dug (unfertilised) plots. Similarly, the extractable P in dug + P plots and in dug + N + P was about six times greater (each case) than in dug (unfertilised) plots. Adding a carbon source (sugar) significantly decreased the level of available NO_3^- - N in disturbed plots compared to dug (unfertilised) plots ($p < 0.05$) with no C (sucrose) added. By comparison, adding a C source (sugar) to dug (unfertilised) plots did not cause a significant change in the level of extractable P (Table 5.3).

Table 5.3. Extractable nutrients (NH_4^+ - N, NO_3^- - N & PO_4^{3-} - P) in each of the treatments, of soil disturbed plots (at 70 days after digging).

Treatments	NH_4^+ - N (μg g^{-1})	NO_3^- - N (μg g^{-1})	total N (μg g^{-1})	P (μg g^{-1})
undisturbed	0.68 (0.22)	0.19 (0.03)	0.87 (0.25)	1.31 (0.11)
dug (disturbed)	12.12 (1.18)	6.90 (0.96)	19.02 (2.14)	2.32 (0 .22)
dug + C (sugar)	11.00 (1.20)	4.50 (0.60)	15.50 (1.80)	2.67 (0.21)
dug + N	35.68 (3.59)	20.29 (1.78)	55.97 (5.37)	2.98 (0.35)
dug + P	15.44 (1.12)	2.74 (0.81)	18.18 (1.92)	15.42 (2.18)
dug + N + P	43.56 (3.46)	25.86 (2.96)	69.42 (6.43)	15.18 (2.15)

Mean values of nutrients are expressed in μg g^{-1} (dry weight of soil), followed by standard errors are in brackets (n = 8).

5.4 Discussion

5.4.1 Comparison of undisturbed plots and disturbed plots (with no nutrients added)

The high relative biomass of exotics (thistles, other broadleaf weeds, annual grasses) observed on soil disturbed plots is typical for disturbed areas in lowland native grasslands (Muir and Carr 1994). The growth of these types of plants has already been linked to elevated nutrient levels in lowland native grassland remnants (Craigie and Stuwe 1992) but the association between soil disturbance, elevated available nutrient levels and rapid dominant growth of exotics has not been made. The elevated nutrient levels measured in soil disturbed plots compared with undisturbed plots (Table 5.3) and the parallel massive increase in biomass of exotic annual grasses and broadleaf weed species is primary evidence that disturbance leads to elevated nutrient levels, which in turn leads to rapid growth of exotic plant species.

5.4.2 Comparison of disturbed plots (no nutrients added) and disturbed plots with nitrogen (N) or phosphorus (P) added alone.

The results of trials involving the addition of nutrients to soil disturbed areas (Fig. 5.2, Table 5.3) support the view that available nutrient levels are limiting for some exotic plant groups growing on disturbed soil, which adds weight to the proposal that the flush of available nutrients into the soil following digging promotes growth of exotics. The results also support the view that phosphorus was more limiting for the growth of annual grasses than nitrogen (Fig. 5.2) but that neither N or P alone was limiting for broad leaf exotics. Average biomass of thistles and other broad leaved species were somewhat higher in P - added plots than N - added plots, but results were not significantly different in these trials ($p > 0.1$). However overall growth of broadleaf species may have been affected by low water availability during the experiment. It was possible that a repeat of this experiment in higher rainfall years might produce a different result.

Soil was analysed for extractable N and extractable P when the biomass samples were taken (Table 5.3). It was observed that the extractable P in P - added plot was six times greater than dug alone (unfertilised) plots. Similarly, extractable N in N-added plots was 3 times greater than dug alone (unfertilised) plots. The average NO_3^- - N level in the P - added treatment was significantly lower ($p < 0.01$) than dug alone (unfertilised) treatment. An explanation for this might be that if P was more limiting in the soil than N, when P was added to the soil, this allowed a significant increase in plant growth and the concomitant uptake of available N from the soil, causing relative N depletion, compared with disturbed only plots, where weed growth and hence N uptake was limited by lower P levels.

5.4.3 Comparison of disturbed plots (no nutrients added) and disturbed plots with Nitrogen (N) and Phosphorus (P) added together

The very high biomass of exotic species on plots to which N + P had been added, compared with N - alone, P - alone and disturbed unfertilised plots (Fig. 5.2), is strong evidence that N and P are limiting for growth of weeds on disturbed sites, and hence that the high levels of N + P released when soil is disturbed is a primary factor causing high levels of weed growth.

The higher biomass of exotic species in disturbed plots to which N + P had been added compared to disturbed plots to which either N or P were added alone was most likely because when N or P were added alone, the other of these two nutrients (P or N) became limiting to plant growth. The increase in growth of exotic annual weeds on N + P plots was not as great as for the broadleaf weeds. This can be explained by the very high growth rates of broad leaved weeds (including thistles) which would have been enough to suppress the growth of annual grasses by utilising above ground (light) and below ground (available nutrients) resources.

5.4.4 Comparison of disturbed alone (no nutrients added) plots and disturbed plots with Carbon (C) added.

The lower total biomass of exotic species (annual grasses and broad leaf species) in disturbed plots to which C had been added than in disturbed alone (unfertilised) plots is likely to be due to lower level of available NO_3^- - N in disturbed and C added plots than in disturbed alone (unfertilised) plots (Table 5.3). The most likely explanation of reduced available nitrogen levels in the soil to which C had been added is the N - demand theory outlined by Brady (1984). According to this theory adding C (sugar) would have increased the C : N ratio of the soil and in doing so would have indirectly increased soil microbial activity. When this ratio is high (ie. a high comparatively high level of organic carbon is present), the heterotrophic soil microorganisms (bacteria, actinomycetes and other fungi) become active and multiply rapidly. Hence, any increase in microbial biomass requires that nutrients be removed from the soil. Paul and Clark (1989) have put forward another theory as to how soil organic matter can affect denitrification, and more specifically the level of nitrate nitrogen in the soil. Most denitrification is accomplished by heterotrophic bacteria, and therefore the process is strongly dependent on carbon availability. Decomposition of organic matter increases the level of CO_2 in the soil and reduces the level of O_2, thus increasing the demand for NO_3^- as an alternative to O_2 as an acceptor of electrons during microbial growth. Paul and Clark (1989) also found that higher levels of easily decomposable carbon promoted higher rates of denitrification. According to both theories, higher organic matter content decreases available nitrate nitrogen in the soil. Results from the experiments involving C addition to disturbed soil in native grasslands (Table 5.3) support these theories because there was significantly lower NO_3^- - N in disturbed plots to which sugar had been added than in disturbed only (unfertilised) plots ($p < 0.01$). In soils, NO_3^- - N is usually more limiting for plant growth than NH_4^+ - N (Paul & Clark 1989). Most plants can more easily absorb NO_3^- than NH_4^+. The NH_4^+ ion in the soil is held on the exchange complex and its movement into and through the soil water is greatly restricted, whereas the readily soluble NO_3^- ion can move into the plant root either by diffusion or by mass flow with water (Paul & Clark 1989). Because the grassland soil at Derrimut were rarely at field capacity, removal of nitrogen via C addition, by mobilization of nitrogen as NO_3^- in mass flow of water is a less likely explanation for C addition effects on lowered soil nitrogen than the model in which added C stimulates microbial growth, in turn removing nitrogen. The lower level of available N in disturbed + C treated plots is a feasible explanation as to why there was a significantly lower biomass of exotic plants (annual grasses and other broad leaved species) on disturbed plus C (sugar) added plots than disturbed alone (unfertilised) plots.

5.4.5. A model for weed invasion following soil disturbance

The high levels of weed growth following soil disturbance in lowland native grasslands have long been a cause of concern and frustration to land managers (for example, see McDougall 1989, Stuwe & Craigie 1992, Scarlett & Parsons 1982 and Kirkpatrick *et.al* 1995) and those attempting restoration of disturbed sites. Because of the high impacts of weed growth on soil disturbed sites in the listed western (basalt) plains community, the action statement (FFG Act 1998 Action Statement No. 53) for this community identifies soil disturbance and associated weed invasion as a key threatening process. Based on the results of trials reported in chapters 3, 4 and 5 of this thesis, it is possible to advance a model for how soil disturbance leads to weed invasion,

1. Growth of exotic annual grasses and broadleaf weeds is suppressed by competitive utilisation by native perennial grasses of light (above ground) and

available nutrients (N & P) that are limiting for exotic weed growth below ground as originally proposed as a hypothesis by Hocking (1996).

2. Physical Soil disturbance lowers or removes dominance by native perennial grasses of above ground competition for light.

3. Soil disturbance also leads to death above ground and below-ground of native perennial grass vegetation.

4. Following the death of native perennial grasses, rapid mineralization (decomposition) of plant material leads to high levels of available nutrients in the soil.

5. The high levels of previously limiting nutrients (N plus P) below ground, and the lack of competition for light aboveground, causes rapid growth of exotic weeds which quickly dominate disturbed sites, limiting the availability of light and nutrients levels for establishment and growth of natives.

If this model is correct, then management of weed growth on soil disturbed sites in native grasslands will require the combined manipulation of access to light (above ground) and levels of available nutrients required for growth (below ground).

It is also likely that different management regimes employed for control of native perennial grass biomass in lowland grasslands (e.g. fire, mowing, grazing) will have specific outcomes for levels of light availability and levels of nutrients that will affect the rates and levels of weed growth. Some of the effects of fire and mowing as management regimes on available nutrient levels in the soil under western (basalt) plains grasslands and the effects in turn of these nutrient levels on weed growth are the subject of the chapters 7 and 8 of this thesis.

Chapter 6

The effects of plot size of soil disturbance on available soil nutrients and weed biomass.

6.1 Introduction

Results reported in chapter four demonstrated that available nutrients (N and P) in soil disturbed plots were much higher than in undisturbed (control) plots after 70 days. The results reported in chapter six investigated the levels of available nutrients (N, P & K) and other ecological related soil parameters (soil moisture content, pH and organic carbon) in large and small soil disturbed plots.

Physical soil disturbance in natural communities ranges from the large scale (eg. hurricane) to very small scale (eg. hoof prints) patches (Whitmore 1978, 1982). The physical characteristics of gaps created by soil disturbance, such as size and shape have been found to affect many of the edaphic and biotic characteristics of gaps. In grasslands most soil disturbance gaps are made by animal activities, including mound building, scraping and digging of borrows, trampling or deposition of large faeces (White 1979). In all of these cases except for the deposition of faeces, there is physical disturbance of the soil. Soil disturbance gaps in forests and the subsequent changes that occur in species richness and distribution have been studied extensively (Denslow 1980 a & b; Oliver 1981; Picket 1980; Whitmore 1978, 1982). Microclimate and related environmental parameters (light, humidity, evaporation, soil moisture) in soil disturbed gaps are determined by many factors. For example, the duration and intensity of light received in a gap depend on its size, shape, slope, orientation, height of surrounding plants or trees and the type of disturbance. Results from several studies (Denslow 1980 a & b; Whitmore 1975) have shown that light intensity and duration are greater in soil disturbance areas than undisturbed area. Soil and air temperature is much higher for soil disturbed gaps and fluctuates over a greater range. Air humidity in disturbed areas is comparatively low, while evaporation from the soil surface is high. But at a few centimetres in depth, soil moisture may be higher in gaps than in adjacent undisturbed areas (Whitmore 1975).

As outlined above, microclimate in soil disturbed gap is affected by the size, shape and the type of disturbance. These changed micro climatic conditions in turn affect soil microbial activity. Consequently, in addition to direct edaphic effects the available soil nutrients in soil disturbed gaps can also be affected by the size of the gaps via effects on rates of decomposition of organic matter, and microbial assimilation of available nutrients. In general, larger soil - disturbed gaps have more favourable microclimatic conditions (temperature, moisture and light) for microbial activity than smaller gaps.

6.2 Methods

6.2.1 Experimental design:

Experimental plots were set up at the Victoria University of Technology, St. Albans campus grassland reserve site (described in chapter one).

Soil was disturbed and plots were set up using the method reported in chapter 4, except that two different sizes of disturbed plots were used.

- 70 cm diameter
- 35 cm diameter

There were eight replicates of each treatment, randomized across a total area of 15 m x 20 m in *Themeda triandra* dominated grassland.

6.2.2 Soil sampling and analysis

At one year after experimental plots had been set up, the available soil nutrients (N, P & K) and other soil related parameters (organic carbon, moisture content and pH) were measured in all plots at 2 - 5 cm soil depth. Soil cores were taken at the center of each plot. The methods for taking soil core samples taken and analysing these for available soil nutrients (N, P & K), organic carbon (C), soil moisture content and pH are described in chapter two - section 2.3.

Standard tests for significance of difference between treatments were carried out using single factor ANOVA.

6.2.3 Plant biomass sampling and analysis

At the same time that soil samples were taken for analysis (December 1995, - one year after soil disturbance) all of the vegetation on each plot was cut to the ground, separated into major plant groups and dried at 70^0C until constant weight was achieved. Plant biomass density was calculated by deviding the dry weight biomass harvested by the area from which it was harvested, as detailed in the methods section of chapter 4.

6.3 Results

The total extractable nitrogen (NH_4^+ - N + NO_3^- - N) and extractable potassium (K) were significantly higher in larger plots ($p < 0.01$) both cases) than in smaller plots (Table 6.1). The availability of phosphorus (P) was not significantly different between larger and smaller plots ($p > 0.1$) (Table 6.1), although the average levels of available P were 18 % lower in small plots compared to large plots. The high variability of P levels in small plots in this instance may have masked differences in available P levels between treatments.

Table 6.1. Extractable soil nutrients (N, P & K) at 2 cm - 5 cm soil depth , between large and small disturbed plots.

Treatment	NH_4^+ - N ($\mu g\ g^{-1}$)	NO_3^- - N ($\mu g\ g^{-1}$)	total N ($\mu g\ g^{-1}$)	PO_4^{3-} - P ($\mu g\ g^{-1}$)	K ($\mu g\ g^{-1}$)
dug large plots	3.89 (0.52)	1.24 (0.10)	5.13 (0.62)	2.18 (0.45)	359.12 (12.57)
dug small plots	3.11 (0.35)	0.76 (0.01)	3.87 (0.36)	1.82 (0.68)	325.00 (10.63)

Mean values are expressed in $\mu g\ g^{-1}$ (dry weight) of soil and standard errors are in brackets ($n = 8$). Soil moisture content in larger plots was significantly lower ($p < 0.01$) than in smaller plots (Table 6.2).

Table 6. 2. Soil moisture contents between large and small disturbed plots.

Treatment	Moisture content / % (air dry weight of soil)
dug large plots	22.76 (1.70)
dug small plots	25.04 (1.30)

The mean values of soil moisture contents and standard errors are given in brackets (n = 8).

The organic carbon (C) content was not significantly different ($p > 0.1$) between larger and smaller plots (Table 6.3).

Table 6. 3. Soil organic carbon (C) between large and small disturbed plots.

Treatment	Organic carbon (C)/ % (dry weight of soil)
dug large plots	7.00 (0.40)
dug small plots	6.60 (0.22)

Mean values organic carbon (expressed as % dry weight of soil) and standard errors are in brackets (n = 8).

The pH was not significantly different ($p > 0.1$) between larger and smaller plots (Table 6. 4).

Table 6. 4 Soil pH between large and small disturbed plots.

Treatment	pH
dug large plots	5.17 (0.06)
dug small plots	5.20 (0.06)

Mean values of pH and standard errors are in brackets ($n = 8$).

Total plant biomass density harvested from the larger plots was significantly higher ($p < 0.01$) than the plant biomass from smaller plots (Fig. 6.1). The difference was mainly due to the annual grass biomass on larger plots being 50% higher than on smaller plots.

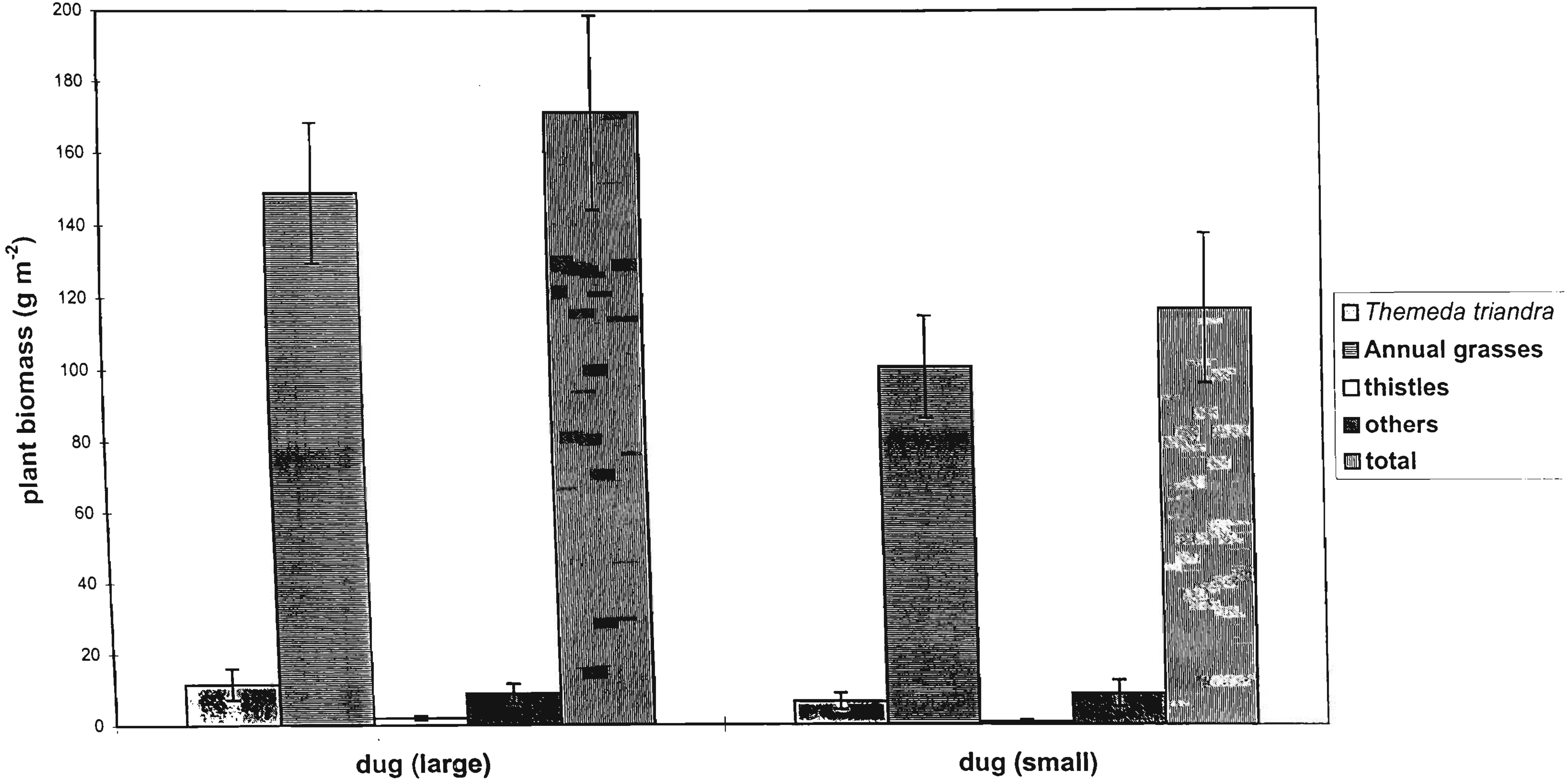

Fig. 6.1. Pant biomass in dug (large) and dug (small) plots Mean values and standard errors (error bars) are shown in y axis.

6.4 Discussion

There are several likely reasons (or combination of reasons) for the significantly lower total extractable nitrogen ($p < 0.01$) and extractable potassium ($p < 0.01$) in small plots than in large plots. The soil and air microclimatic conditions in smaller soil disturbed gaps were highly likely to be different from those in larger gaps as indicated by the different soil moisture content levels (Table 6.2). Smaller gaps would have had a higher percentage of the disturbed area shaded by surrounding plants. Therefore, less light and heat are likely to have reached the soil surface. As a result the small gap environment would have tended to be cooler with higher moisture content in the soil. The micro climatic conditions in smaller gaps were probably less favourable than in larger gaps for decomposing microbial activity. Therefore, less organic matter decomposition might have been occuring in smaller gaps than larger gaps. The available light intensity at the soil surface would also have affected atmospheric nitrogen (N_2) fixation (Knapp & Seasted 1986). In addition, over one year following disturbance, Kangaroo Grass and other deep rooted perennial plants on the boundary of each disturbance plot would have undergone regrowth of their roots into the disturbed soil, which would have extracted soil nutrients. This effect would have been comparatively greater on available soil nutrient levels in small plots than in large plots, because the total boundary distance to area ratio for small soil disturbance plots is larger than for larger plots. A combination of the effects described above would have led to lower levels of total available nitrogen (NH_4^+ - N & NO_3^- - N) and potassium (K) in smaller gaps than in larger gaps.

Soil analytical results showed that the extractable phosphorus (P) was not significantly different ($p > 0.1$) between large and small plots. There were several reasons for this. According to Bonis *et al.* (1997) for plants invading a soil disturbed gap, the primary limiting nutrient at first is phosphorus (P). Therefore it is possible that most of the available phosphorus (P) released as a result of mineralization had been taken up by plants by the time that the assessment were undertaken (after one year). The analysis of pH of soil showed that pH was not significantly different between ($p > 0.1$) small and large plots (Table 6.4). As a result the extractable phosphorus content in the soil should not have been significantly different between small and large plots. Another possible explanation for the lack of difference in extractable P levels between plot size treatments is that mineralized phosphorus was accumulated quickly by soil micro-organisms. Finally, it is possible that the high variability in extractable P levels in the particular plots in this set of trials masked any difference in P levels between treatments. Overall, despite no statistically significant difference in extractable P levels between treatments, the average extractable P levels in small dug plots were 18 % lower than in large dug plots. If this experiment were repeated in another year, or at another site, or perhaps with a larger number of replicates, it is possible, given the trend towards lower average P in small dug plots that a significant difference between treatments for extractable P might be found.

The effects of the different available nutrient levels in small and large soil disturbed plots was paralleled by different biomass densities of plants (Fig. 6.1), with a much higher total biomass density of weeds on large plots compared with small plots. These results are consistent with the outcomes of the nutrient addition experiments described in chapter five, which demonstrate clearly that N and P were limiting for weed growth in soil disturbed plots, and consequently that nutrients release following soil disturbance would have promoted the of exotic annual grasses and broadleaf species.

Chapter 7

The effects of nutrient addition on the above ground vegetation (weeds and native plants) in recently burnt grassland plots.

7.1 Introduction

It is well documented that biomass reduction (fire, grazing and mowing) changes the diversity and composition of plants in native grasslands across a range of grassland types worldwide (see chapter one). Nutrient levels have also been implicated in determining native grassland composition and biomass at any given time. Several studies have found that fertilizer addition reduces plant diversity and increases weediness in native grasslands (Willis and Yemm 1961; Jeffrey *et al.* 1993)

Some links between burning, soil nutrient levels and plant composition have been established. Aldous (1935), Hulbert (1973) and Bragg and Hulbert (1976) have all pointed out that prescribed burning is necessary to maintain high production levels and keep out woody species in grassy ecosystems. Cook (1965) proposed that in perennial grasslands, burning may lead to either increases or decreases plant diversity, but in annual grasslands the effect is usually to reduce the diversity. Fire commonly favours forbs over grasses in both annual grasslands (Bently & Fenner 1958; Biswell 1956) and perennial grasslands (Daubenmire 1968). Gibson *et al.* (1993) showed that the combination of burning and fertilizer addition increased C_4 grass cover in the tall grass prairie of Kanzas, USA. They also found that the percentage cover of woody species decreased after burning.

Chapter three of this thesis reported on the results of trials showing that available nutrient levels were low in study sites with intact soils. In chapter four it was demonstrated that soil disturbance leads to increased available soil nutrient levels, most likely as a result of increased mineralization of plant material killed by digging, and this in turn leads to increased weed growth. Also when nutrients (N + P) were added to plots with disturbed soil, higher levels of weed growth were observed than in disturbed-alone plots (chapter five).

After an autumn burn (April 1996) conducted as part of this study on trial plots at Derrimut grassland which had not been burnt for five years, a significant number of annual weeds were observed growing on plots in spring. This was possibly due to a type of "detritus effect" in long - unburnt grasslands (Knapp & Seastedt 1986). According to this theory (detritus effect), accumulation of detritus (dead plant materials above and within the plant canopy) in long unburned grasslands decreases plant growth rates and productivity. Roots of plants are not as effective in uptake of nutrients as open canopy (burned) grasslands. As a result, the amounts of nutrients taken up from the soil decline and nutrients accumulate in the soil. The net effect of detritus is the build - up of available nutrients (primarily N and P) in the soil in western (basalt) plains native grasslands. See chapter 8 for evidence of nutrient accumulation in long unburnt grassland.

Despite the build-up of nutrients in long-unburnt grasslands, the growth of new seedlings which might take advantage of elevated nutrient levels, such as annual weeds, would be suppressed in long unburnt grassland because of canopy closure of dominant perennial grasses. However, when the canopy is removed, for example by fire, a rapid increase in biomass of seedlings of these types of plants should result.

The opportunity arose to investigate how annual grasses and other weeds respond to changes in nutrient levels in grasslands with undisturbed soil. The plan was to burn, in autumn of 1997, small plots in long unburnt areas, and add nutrients to sub plots, so as to determine what influence levels of available nutrients had on growth of weeds and native species. Recently burnt grasslands were chosen for the study to minimise the effects of above ground competition (ie. canopy cover) between natives and weeds, and highlight on the growth responses attributed to added nutrients, in the absence of soil disturbance.

It was hope that such an investigation would provide insights into the role that available soil nutrients play in contributing to the weediness of intact native grassland remnants.

7.2 Methods

7.2.1 Design of experimental plots

The study was conducted at Derrimut grassland reserve. Eight randomized replicate blocks of 5 m x 2.5 m across a total are of 75 m x 20 m were marked on the ground and burnt in autumn (May) 1997. Each block was divided into five treatment sub blocks (2.5 m x 1 m). Within each sub block the actual monitoring of effects was conducted in randomized replicate 1 m x 0.75 m (plot) areas, so as to leave a buffer zone around each monitored quadrat and minimise edge effect (Fig. 7.1).

The five quadrats in each block were randomized and each was treated with one of following treatments, beginning in spring (October) 1997:

- control (burnt only).
- burnt and nitrogen (N) added (0.832 g m^{-2} $month^{-1}$ of N as NH_4NO_3).
- burnt and phosphorus (P) added (0.832 g m^{-2} $month^{-1}$ of P as triple super phosphate).
- burnt and nitrogen & phosphorus (N + P) added (0.832 g m^{-2} $month^{-1}$ of N as NH_4NO_3 and 0.832 g m^{-2} $month^{-1}$ of P as triple super phosphate).
- burnt and sugar (C source) added (3.32 g m^{-2} $month^{-1}$ of C as sucrose).

Nutrients were hand broadcast on to experimental plots monthly until end of spring for 3 months. Adjacent, randomized unburnt blocks were also monitored in the same way as burnt and treated plots.

7.2.2 Biomass analysis.

After three months of growth over spring the number of plants of each species was separately counted. Each plant was clipped at ground level and collected by species. The plant materials were dried at 70^0C until samples attained constant weight and the final weight was recorded.

experimental plots

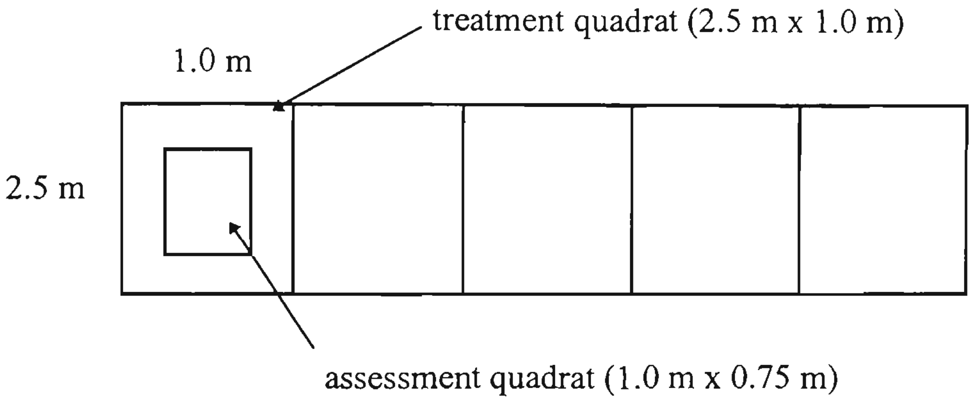

Fig. 7.1. Arrangement of treatment and assessment quadrats, within each of eight replicated burnt areas.

7.2.3 Statistical analysis

ANOVA multiple comparison test was performed. Details of statistical analysis are summarised in appendix 3.

7.3 Results

In early summer (January) 1998 the sampled biomass of unburnt plots was made mainly up of *Themeda triandra* grass (> 95%). By comparison in plots burnt the previous Autumn (May) the average biomass of *Themeda triandra* grass was much lower at about 40% (Fig. 7.2). The rest of the plant biomass in burnt plots was mainly (> 95%) weedy species (annual grasses and broad leaved thistle sp.).

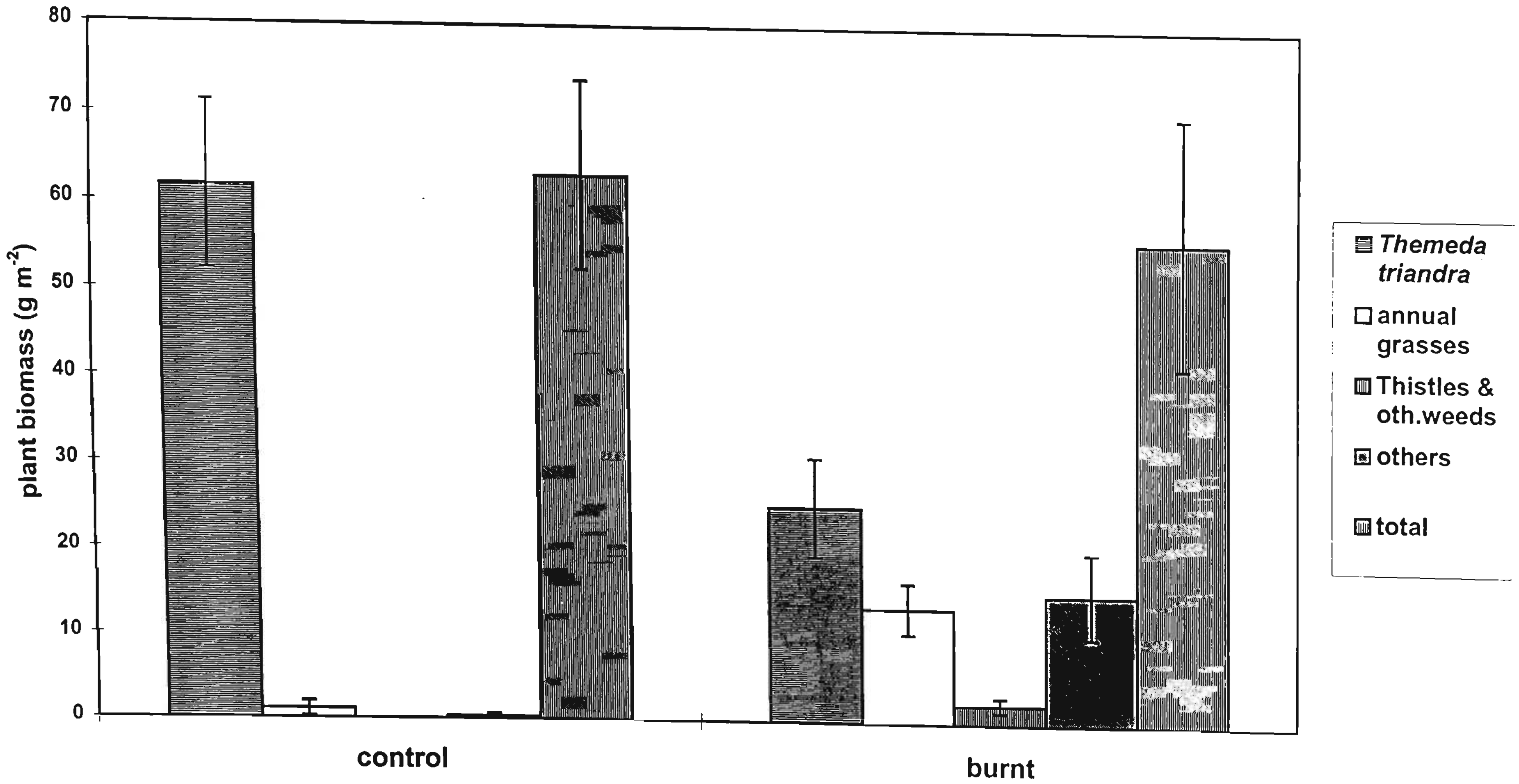

Fig. 7.2. The above ground biomass of major plant groups in control (unburnt) and burnt plots in January 1998, eight months after burning. Mean values and standard errors (error bars) are shown in Y axis.

Similar plant species were observed across all treatments in burnt only and burnt - plus - nutrient addition plots. Species composition was made up mostly annual grass weeds (*Briza maxima, Briza minor, Vulpia bromoides* and *Aira caryophylla*) and broadleaf weeds (*Picris echioides, Sonchus oleraceous, Cirsium arvense* and *Hypochoeris glabra*) in addition to *Themeda triandra* grass (Fig.7.3). The analysis of biomass showed that the native *Themeda triandra* and other exotic weedy species were not significantly different across burnt, burnt plus Nitrogen (N) and burnt plus phosphorus (P) plots ($p > 0.1$ all cases). The average biomass of weedy annual grasses and broad leaved weed species (mostly thistles) was significantly lower ($p < 0.01$ each case) in plots which received sugar than in control (burnt only) plots (Fig. 7.3). The addition of a carbon (C) source (sugar) had no greater effect on the biomass of *Themeda triandra* grass.

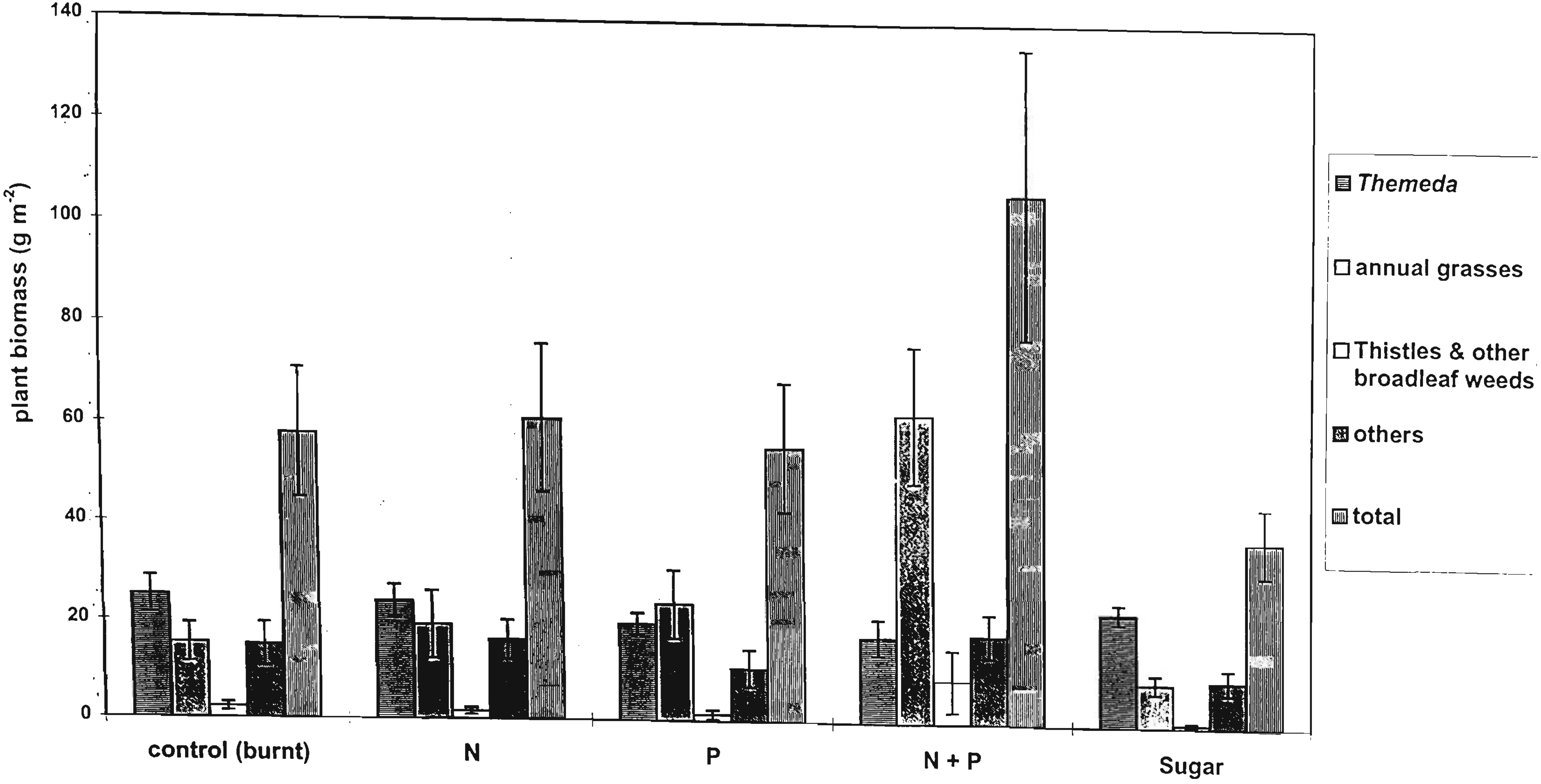

Fig. 7.3. The above ground biomass (*Themeda* grass, annual grasses, thistles and other broad leaved weeds and other plants) of burnt and nutrient addition plots. Mean values and standard errors (error bars) are shown in Y axis.

The addition of phosphorus (P) to burnt plots caused a significant increase in particular in the biomass of *Vulpia bromoides* (annual grass) in burnt plots (Fig. 7.4). The average biomass of *Vulpia bromoides* was about two times greater in phosphorus (P) added plots than in control (burnt) plots. The addition of N alone did not significantly increase the biomass of any of the annual grass species present. However the average biomass of *Briza maxima* and *Vulpia bromoides* were significantly increased ($p < 0.01$ both species) by adding nitrogen (N) and phosphorus (P) together (Fig. 7.4). The effects of N addition and P addition appeared to be cumulative on the average biomass of *Vulpia bromoides*, because the average biomass of *Vulpia bromoides* in N + P addition plots was approximately 50% higher than in P - alone addition plots.

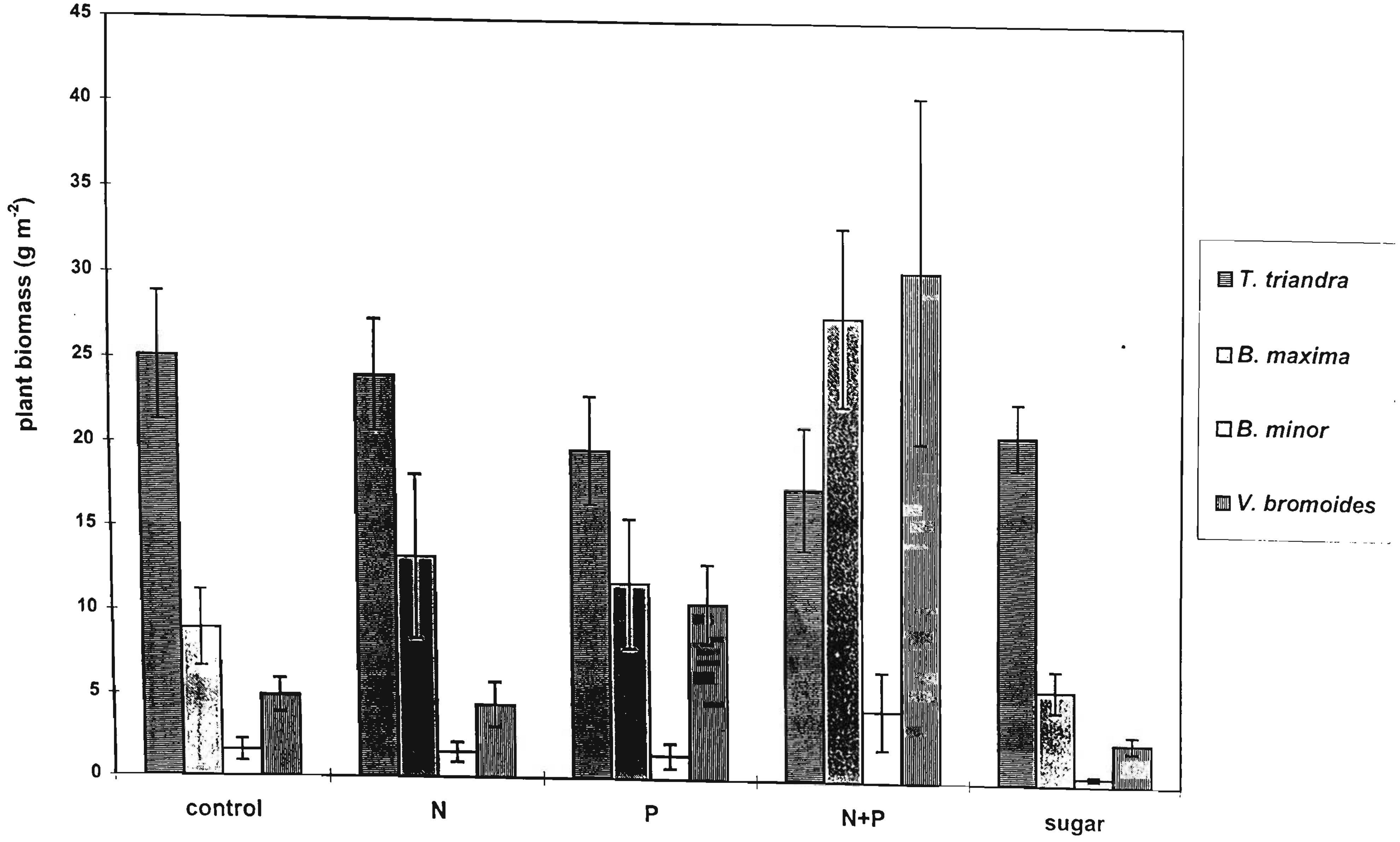

Fig. 7.4. The above ground biomass of *Themeda triendra* and annual grasses (*Briza maxima, Briza minor* and *Vulpia bromoides*) in burnt and nutrient addition plots. Mean values and standard errors (error bars) in Y axis.

The addition of nutrients in any combination did not significantly affect the number of individual plants of each species on the experimental plots (Fig. 7.5). Apparently nutrient addition caused an increase in the size of plants, rather than their number.

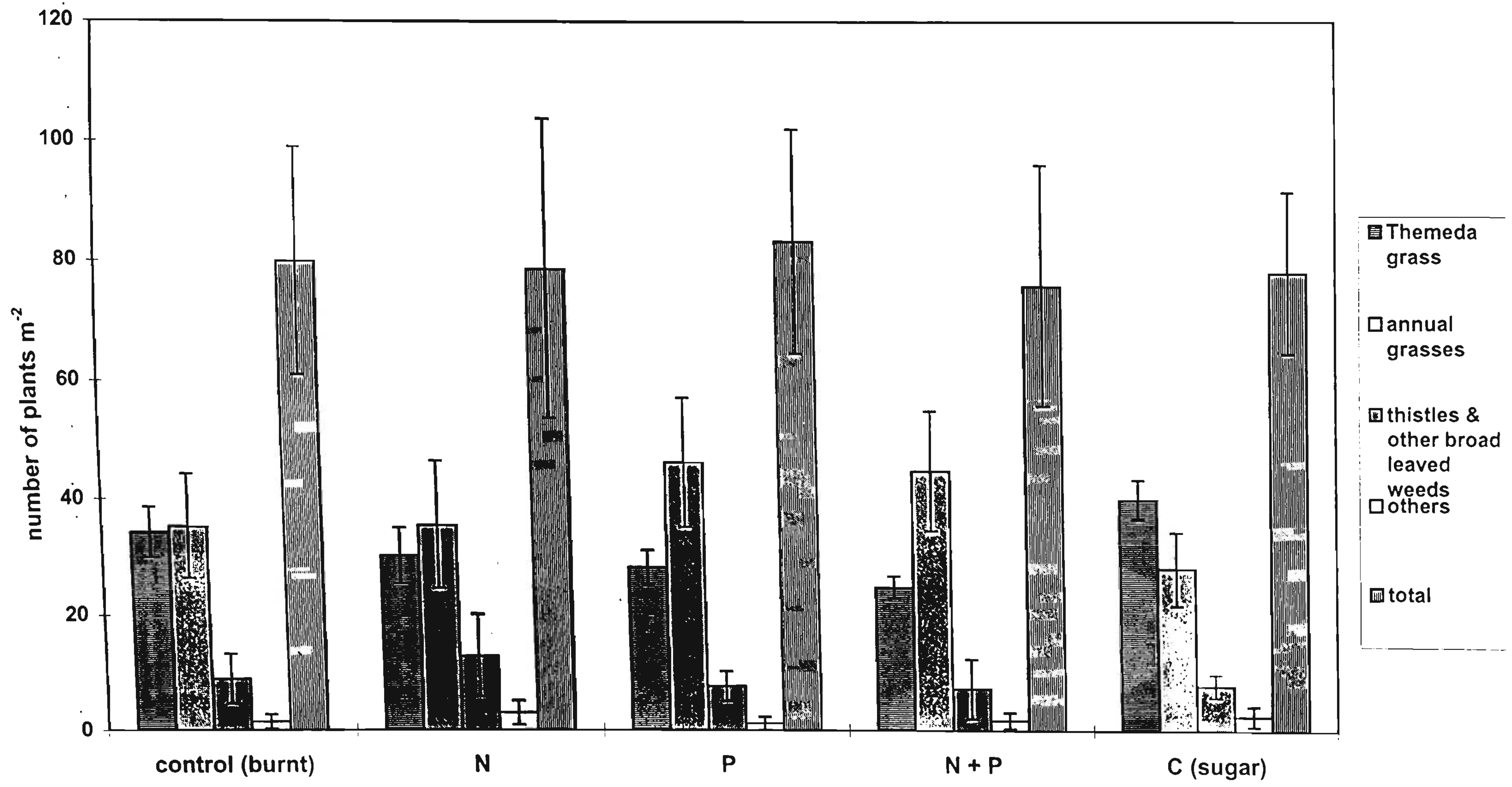

Fig. 7.5. Number of plants (*Themeda* grass, annual grasses, thistles and other broad leaved weeds and other plants) in burnt and nutrient addition plots. Mean values and standard errors (error bars) are shown in Y axis.

The increase in size of plants with nutrient addition is made clearer when the total biomass of each major plant group is divided by the number of plants in the group, as an estimate of average plant biomass (Fig. 7.6).

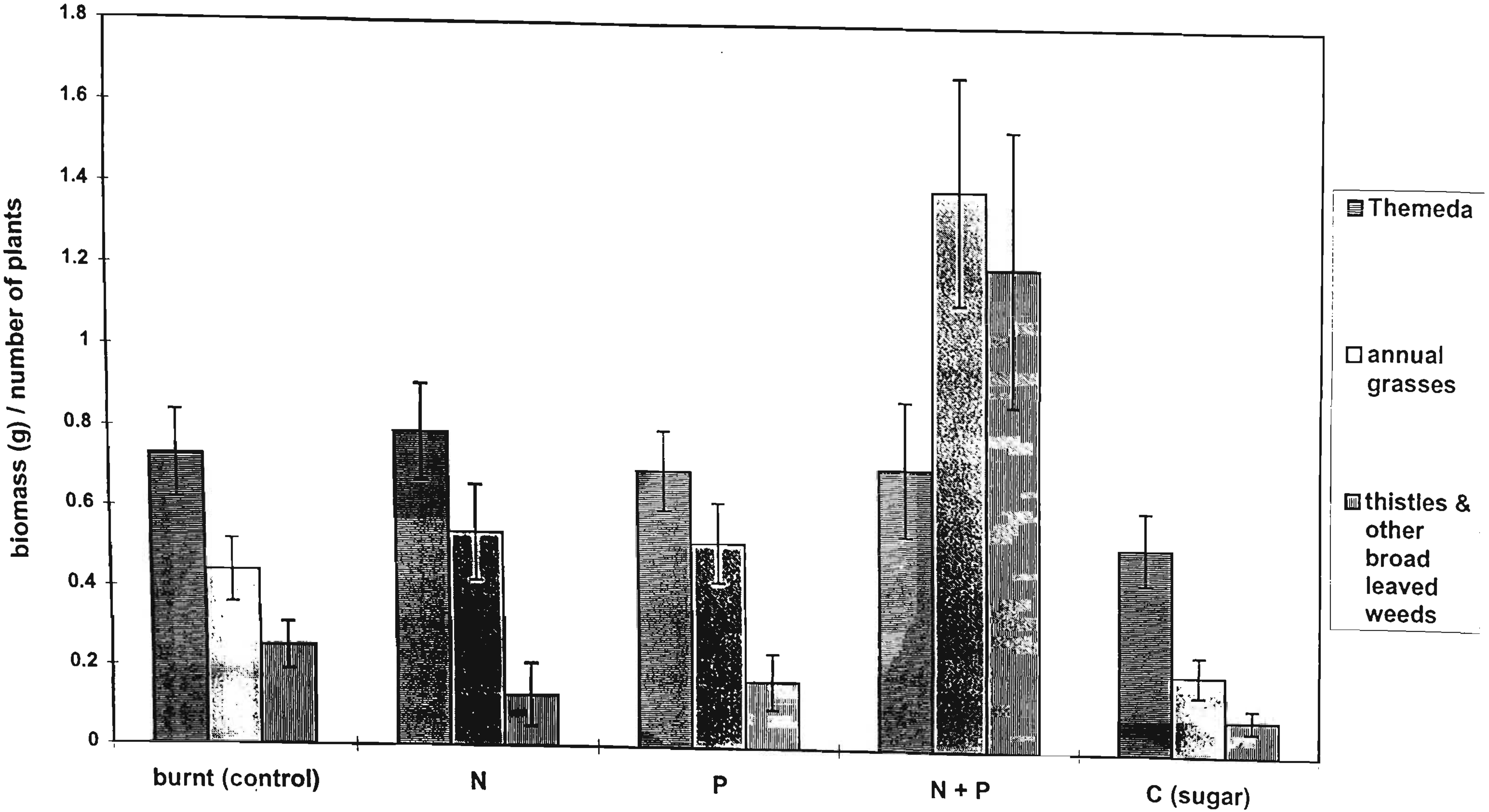

Fig. 7.6. The ratio of biomass / number of plants (*Themeda* grass, annual grasses, thistles and other broad leaved weeds and other plants) in burnt and nutrient addition plots. Mean values and standard errors (error bars) are shown in Y axis.

7.4 Discussion

The higher biomass of exotic annual grasses and broadleaf weed species in recently burnt plots is consistent with the theory of detrital nutrification in long unburnt grasslands and the subsequent effect of increased light reaching the soil as a result of burning, leading to opportunities for growth of highly competitive weedy species over native species (see chapter 8 for discussion of changes over time in soil nutrient levels under unburnt grasslands). Burkes & Grime (1996) pointed out that for limestone grasslands in the United kingdom, exotic species showed an enhanced ability to capture the elevated quantities of light and mineral nutrient resources compared to the indigenous species. Grime *et al.* (1988) also proposed that, in general, indigenous or native species are commonly more slow growing than exotics for which faster growth rates are associated with invasive success.

The effects of adding nitrogen (N) and phosphorus (P) alone and the combination of nitrogen and phosphorus on plant growth varied significantly. The addition of nitrogen (N) and phosphorus (P) alone had no significant effect on either the biomass or number of plants of most plant groups (Fig. 7.3, Fig. 7.5). However the higher biomass of *Vulpia bromoides* in P addition treatments suggests that phosphorus (P) is limiting for growth for *Vulpia bromoides* in recently burnt plots.

The addition of nitrogen (N) and phosphorus (P) together had major effects on the biomass production of several annual grass species. The biomass of exotic annual grasses *Briza maxima* and *Vulpia bromoides* in nitrogen (N) plus phosphorus (P) added plots were significantly higher than in all other treatments (Fig. 7.4). Ford (1997) has obtained similar results for annual grasses in nutrient addition plots (N + P) in a more limited study at Victoria University, St Albans campus grassland reserve. The average biomass of native *Themeda triandra* grass in N alone and P alone plots, was relatively high compared with biomass of *Themeda triandra* in plots receiving both nitrogen (N) and phosphorus (P) together (although not significantly different at $p = 0.05$). This is a possible indication that growth of native *Themeda triandra* grass might have been suppressed to some extent by the high cover of annual grasses in

N + P addition plots, especially because several studies of the effects of nutrient addition to *Themeda triandra* dominated pasture (Groves & Williams 1981) suggest that growth of *Themeda triandra* is promoted by fertilizer addition. The biomass and number of plants of thistles and other broad leaved weeds were not significantly different ($p > 0.1$ both cases) between control (burnt) and burnt and N plus P treatments. The biomass per plant of thistles and other broad leaved weeds in plots received both N and P was about six times greater than in control (burnt only) plots (Fig. 7.6) and was significantly higher ($p < 0.001$). However the number of plants m^{-2} of thistles and other broad leaved weeds (Fig. 7.5) was not significantly different ($p > 0.1$) between burnt (control) plots and burnt and N plus P plots. These results taken together suggest that the addition of N + P does not lead to an increase in the number of annual grass and broadleaf weeds, but rather an increase in their size. The reduction of weedy annual grasses and broad leaved weed species in C (sugar) addition plots was probably because the addition of C decreased available N in the soil (as explained in chapter 5). The results of N + P addition treatments demonstrate that both of these nutrients are limiting for growth of broadleaf weeds. Low levels of either of these would have a negative effect on growth. The relatively low level of available nitrogen (N) in the soil of burnt only plots is likely to have suppressed the growth of exotic weedy species, even if phosphorus levels were not limiting.

The out comes of these results suggest that soil nutrient levels in recently burnt plots play a significant role in determining the size of native grasses, annual grasses and broad leaf weeds and affect the capacity for growth by weeds in intact native grassy ecosystem remnants.

Chapter 8

The effects of burning and mowing on available soil nutrients

8.1 Introduction

In previous chapters it was demonstrated that the levels of the available soil nitrogen (N) and phosphorus (P) in the soil of intact native grassland remnants strongly influence the biomass production of annual grasses and broadleaf weeds. Higher biomass of weeds were observed in treatment plots with artificially higher levels of available nitrogen and phosphorus in the soil (see chapters five and seven). The objectives of the investigations reported in this chapter were to determine the effects of grassland management practices (burning and mowing) on available soil nutrients in different seasons, and to observe whether differences in available nutrient levels with different management regimes were associated with differing biomass levels of weeds.

Previous studies of the effects of burning on native grasslands burning have found that available soil nutrients after fire were decreased in some grasslands (eg. tall grass prairie in U S A) and were increased in other grasslands (eg. snow - tussock grasslands in New Zealand) - see the detailed review in section 8.1.1 below.

By comparison with the number of studies of soil nutrients in native grasslands following burning, few studies have been undertaken to determine levels of available soil nutrients after mowing. Many of the detailed studies of effects of biomass reduction of grasslands (burning, mowing or grazing) were in tall grass prairie remnants in the U. S. A. It appears that no recent studies have been reported which investigate the effects of grassland management practices on available soil nutrients in Australian temperate lowland grasslands. The investigations reported in chapter eight was undertaken at Derrimut and Laverton grassland reserves, at the experimental sites described in chapter one.

8.1.1 Effects of burning on soil nutrient levels

Ross *et al.* (1997) found higher levels of available nitrogen (N) and phosphorus (P) in burnt plots than in unburnt plots 2 years after burning in snow-tussock (*Chionochloa rigida*) grassland in New Zealand. On the coastal plains of southeastern North America, frequent burning of savanna grass raised the total nitrogen (N) of the upper 10 - 15 cm of the soil profile (Garren 1943). Also, burning of the *Boutelona* steppe of south eastern Arizona usually increased the nitrogen (N) content of the soil, even though it had no consistent effect on the total organic matter (Raynolds & Bohning 1956).

Kucera and Ehrenreich (1962) suggested that the removal of large quantities of carbonaceous material in burning might reduce the C/ N ratio of the soil, making nitrogen (N) more available. Trabaud (1994) showed that fire can provide a natural means of recycling nutrients locked up in above ground biomass back into the soil. Trabaud's (1994) showed that burning released some amounts of readily soluble nutrients, such as phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) in ash. Before fire these nutrients were bound in plant live and dead tissues and were unavailable for plants growth. Christenesen (1973) suggested that soil heating and combustion can transform organic nitrogen (N) into readily available forms (NH_4^+ & NO_3^-) for plant growth through mineralization following plant tissue death.

Seasted *et al.* (1991) found that the available nitrogen (N) in burnt plots was significantly lower than the available nitrogen (N) in unburnt plots in tall grass prairie. Ojima *et al.* (1994) proposed that for tallgrass prairie significant amount of nitrogen (N) in plant detritus can be lost to the atmosphere when this material is burnt. Knapp and Seasted (1986) found that fire volatilises about 1.2 to 2.0 g m^{-2} $year^{-1}$ of nitrogen (N) from prairie litter. Risser and Parton (1982) hypothesised that, unless this difference is compensated for by increased nitrogen (N) fixation on burnt sites, annual burning will eventually deplete soil organic nitrogen content. Ojima *et al.* (1994) proposed that in the short term, fire in the tall grass prairie would enhance microbial activity, increase both above and below ground plant production and increase nitrogen use efficiency. They also proposed that repeated annual burning would result in greater inputs of low quality plant residues causing a significant reduction in soil organic nitrogen (N), lower microbial biomass, lower nitrogen (N) availability and higher C:N ratio in soil organic matter.

Ojima (1987) found lower rate of mineralization of nitrogen in burnt plots than unburnt plots in tallgrass prairie. Allen (1964) and Marrs (1993) have shown that potassium (K) was leached rapidly after burning and phosphorus (P) was fixed, rendering both phosphorus (P) and potassium (K) less available for plants in the short term in grasslands.

8.1.2 Effects of mowing on soil nutrient levels

The levels of available nutrients following mowing have not been studied as extensively as the available nutrient levels following burning. Most of the studies of mowing examined its effects on plant diversity. Petgal (1987) found that hay cropping of nature reserves in Holland depleted soil nutrients. The water extractable phosphorus (P) was reduced to an apparent equilibrium value within two years after cutting of grassland and levels of extractable potassium (K) dropped slowly during that period. Total nitrogen (N) changed very little in the same period. Gough and Marrs (1990a, b) and Wells (1980) showed that continuous clipping of natural grasslands in England reduced both the extractable phosphorus (P) and the magnesium (Mg) content in the soil.

8.1.3 Implications of previous studies for investigations of available nutrient levels in lowland grasslands.

It is apparent from the literature reviewed above that there is no consistent linear trend in either available or total N or P levels in soils under native grasslands following burning or mowing. However this does not mean that there will be some more complex non-linear trend of the system were reviewed over a long period of time. The variety of responses is probably a reflection of the complexity of the systems under investigation, the variety of ecosystem components that are affected simultaneously by burning or mowing, and the interactions between grassland ecosystem components which affect available nutrient levels. For example, burning of lowland native grasslands removes the overburden of dead grass (Lunt & Morgan 1998), stimulating new growth of grass which would presumably lower available nutrient levels (via uptake into new shoots). Burning simultaneously exposes the soil to increased sunlight, water filtration and changed temperature and moisture regimes, which would affect leaching of nutrients and rates of nitrogen fixation in as yet unexamined ways.

It is also apparent from investigations reported in chapters 4 and 5 that much of the potentially available nutrients in lowland native grasslands are locked up in the plant tissue, resulting in very low extractable nutrient levels in the soil, and that nitrogen and phosphorus can become quickly available if the plant tissue dies. Management actions which lead to plant death would therefore presumably result in rapid increases in available soil nutrients. Detecting these rapid fluxes, however, may not be easy to achieve. For example, in relatively intact lowland grassland remnants, low level plant death which results in the release of nutrients may not register in monitoring programs designed to measure extractable nutrient levels over time if the surviving plants rapidly take up these released nutrients as soon as they become available.

8.2 Methods

8.2.1 Site preparation : Forty plots each measuring (2.5m x 2.5m) were marked on the ground at each of two grassland sites, Derrimut and Laverton grassland reserves. These plots were randomly allocated to five different treatments, with eight replicates in each treatment.

Treatments :

annually burnt in autumn
control (unburnt / unmown)
burnt once in autumn
annually mown in autumn
mown once in autumn

Burning

All "burn" treatment plots were burnt in April 1996. Before burning, plots were enclosed in "steel boxes" (2.5 m x 2.5 m x 0.5 m) and sprayed with water outside the box perimeter to prevent fire from escaping to the surrounding. Then the plot (area inside the steel box) was burnt. The burnt area was lightly sprayed with water following the burn, before removing the steel box.

Mowing

All "mown" treatment plots were mown in April 1996. Plots were mown with "sickle bar mower" and mown vegetation was removed from each plot by raking.

8.2.2 Soil sampling and analysis

Soil samples were collected and analysed for soil nutrients and other related parameters (moisture, pH and organic carbon (C)) at each of the three active growing seasons autumn (April - May), spring (October- November) and summer (January - February) at both experimental sites Derrimut and Laverton grassland reserves. The detailed methods for soil sampling and analysis were described under chapter 2 (section 2.3).

8.3 Results

The moisture content of plots across treatments and seasons are shown in Fig. 8.1 (Derrimut grassland reserve) and Fig. 8.2 (Laverton grassland reserve). Soil moisture content in burnt plots (annually and burnt once) was significantly lower ($p < 0.001$) than control plots at all seasons, except autumn 1997. Similar results were obtained at each of the grassland sites, Derrimut and Laverton grassland reserves.

The moisture content in mown plots was often less than in control plots but more than in burnt plots. Whether or not moisture content in mown plots was lower than in control plots was affected by season of sampling. The soil moisture content in spring always lower in mown plots than in control plots at Derrimut grassland reserve but this was not always lower (spring 1998) at Laverton grassland reserve.

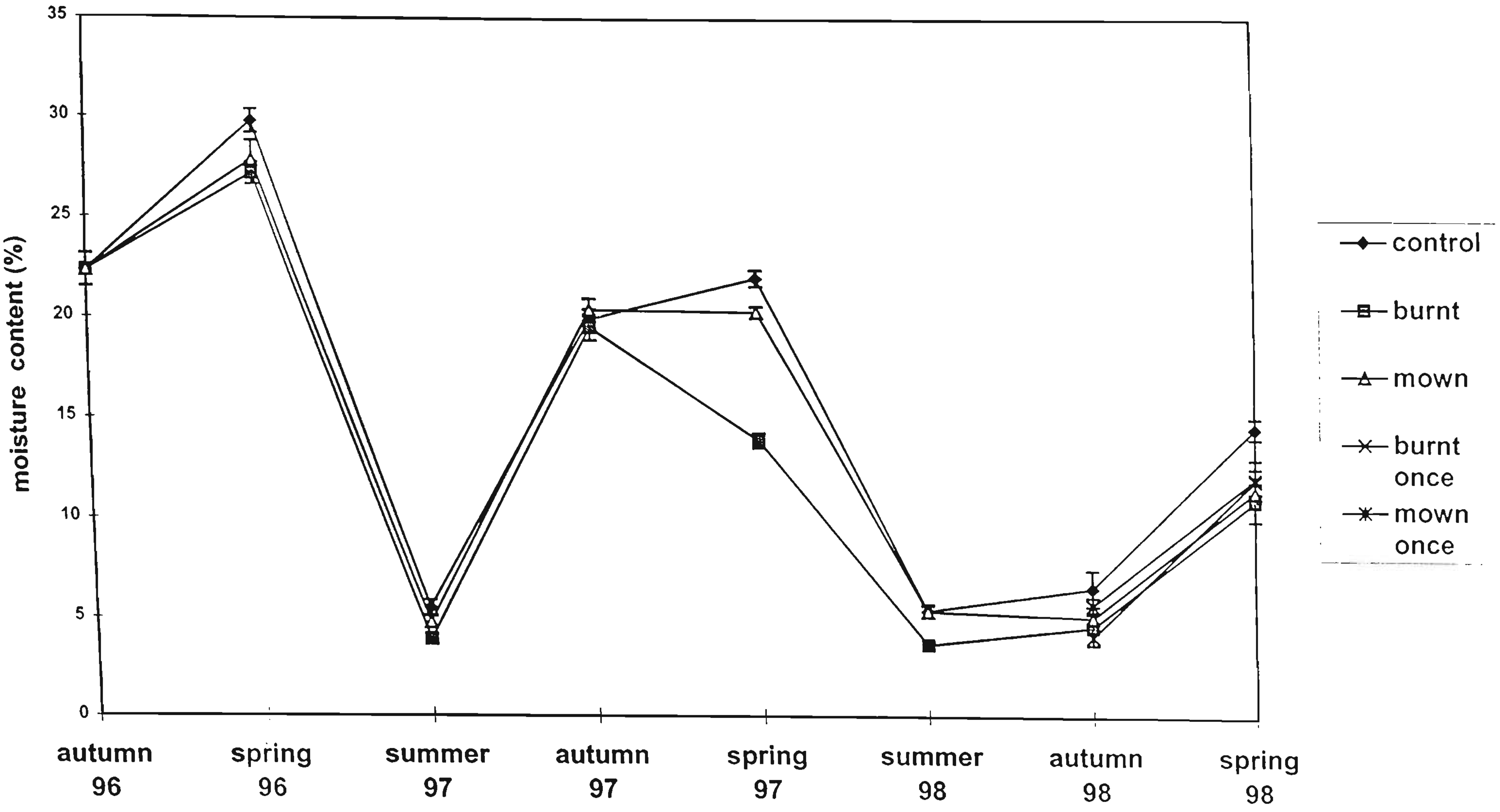

Fig. 8.1. Seasonal variation of moisture in control, burnt (annually burnt and burnt once) and mown (annually mown and mown once) plots from 1996 to 1998 at Derrimut grassland reserve. Burnt once and mown once treatments were only assessed for moisture content in autumn and spring of 1998 (n = 8).

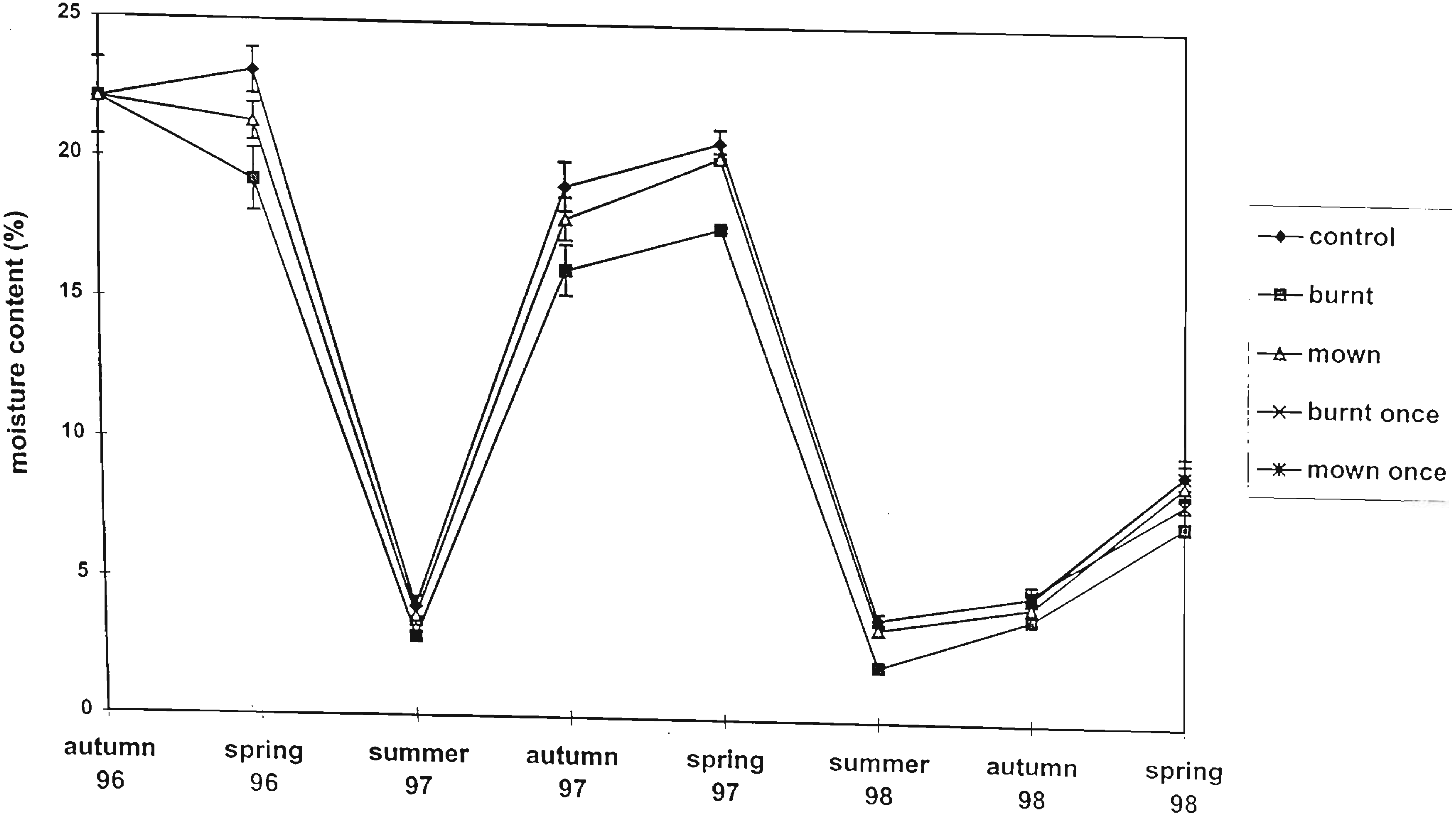

Fig. 8.2. Seasonal variation of moisture in control, burnt (annually burnt and burnt once) and mown (annually mown and mown once) plots from 1996 to 1998 at Laverton grassland reserve. Burnt once and mown once treatments were only assessed for moisture content in autumn and spring of 1998 (n = 8).

The variation of organic carbon (C) content in the soil across treatments and different seasons are shown in Fig. 8.3 (Derrimut grassland reserve) and Fig 8.4 (Laverton grassland reserve). Organic carbon (C) in annually burnt plots was significantly lower ($p < 0.01$) than control plots at both grassland sites (Derrimut and Laverton grassland reserves). Mown plots also sometimes exhibited significantly lower soil organic carbon content than control plots, although this was not consistent across seasons.

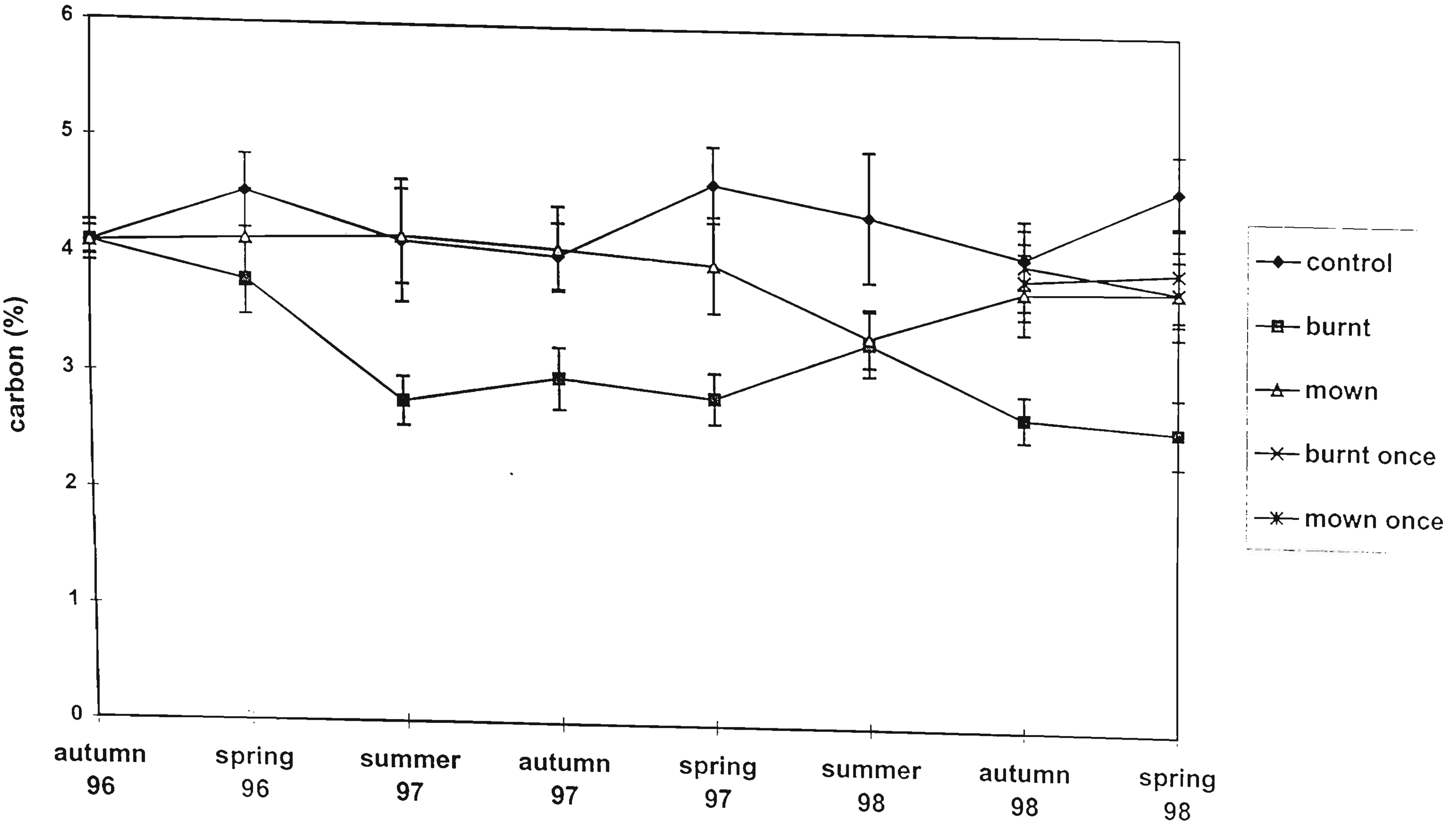

Fig. 8.3. Seasonal variation of organic carbon (C) in control, burnt (annually burnt and burnt once) and mown (annually mown and mown once) plots from 1996 to 1998 at Derrimut grassland reserve. Burnt once and mown once treatments were only assessed for organic carbon in autumn and spring of 1998 (n = 8).

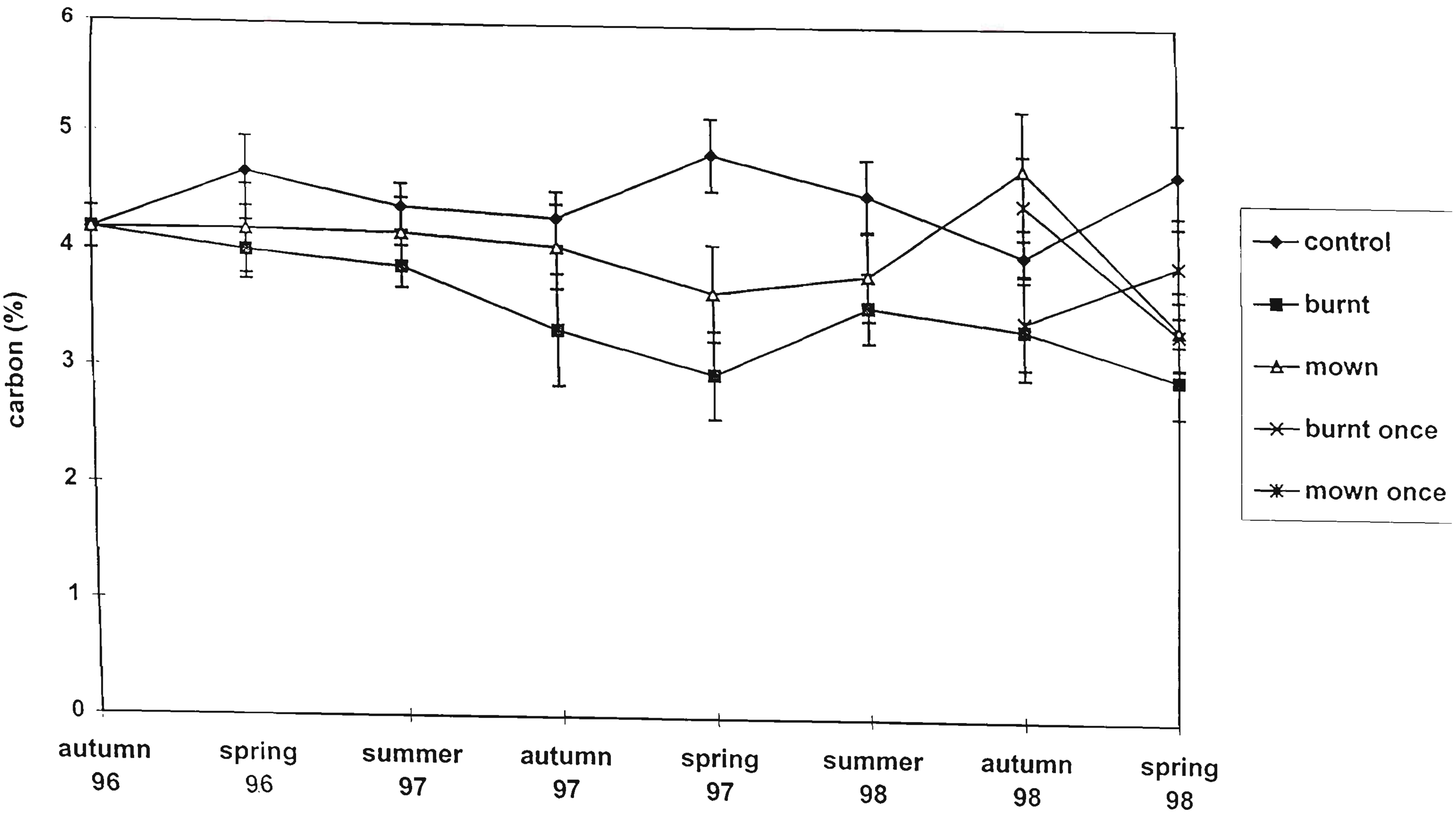

Fig. 8.4. Seasonal variation of carbon (C) in control, burnt (annually burnt and burnt once) and mown (annually mown and mown once) plots from 1996 to 1998 at Laverton grassland reserve. Burnt once and mown once treatments were only assessed for organic carbon in autumn and spring of 1998 (n = 8).

The total levels of soil nutrients, nitrogen (N), phosphorus (P) and potassium (K) are not significantly different between control (unburnt), burnt (annually and burnt once) and mown (annually and mown once) treatments ($p > 0.1$ all cases) in March 1998, two years after treatments had been implemented. Results are shown in Fig. 8.5 to Fig. 8.10. Similar results were obtained at each of the grassland sites (Derrimut grassland reserve and Laverton grassland reserve). However, levels of total N, P and K were all significantly lower in autumn 1998 than in autumn 1996, prior to commencement of treatments. This would have been possibly due to losses of nutrients from the soil by leaching after plant death, during the drier periods in summer 1997 and 1998.

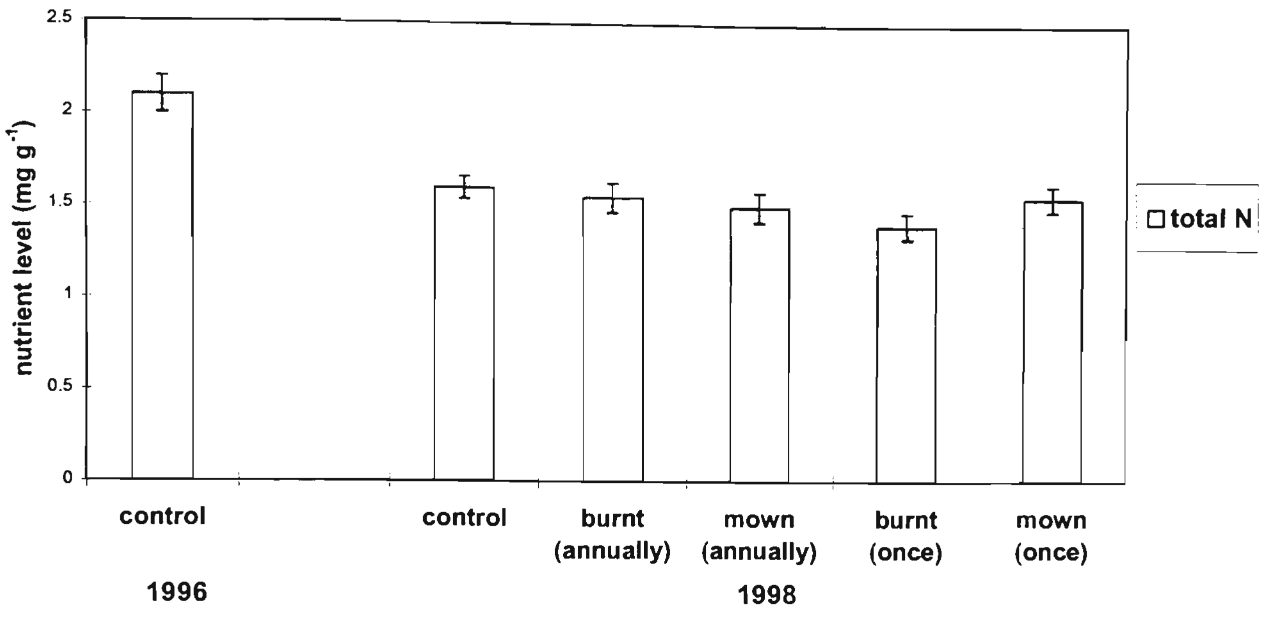

Fig. 8. 5. Total levels of N in control plots (1996) and control, burnt (annually burnt and burnt once) and mown (annually and burnt once) plots (1998) at Derrimut grassland reserve. Bars indicate standard errors (n = 8).

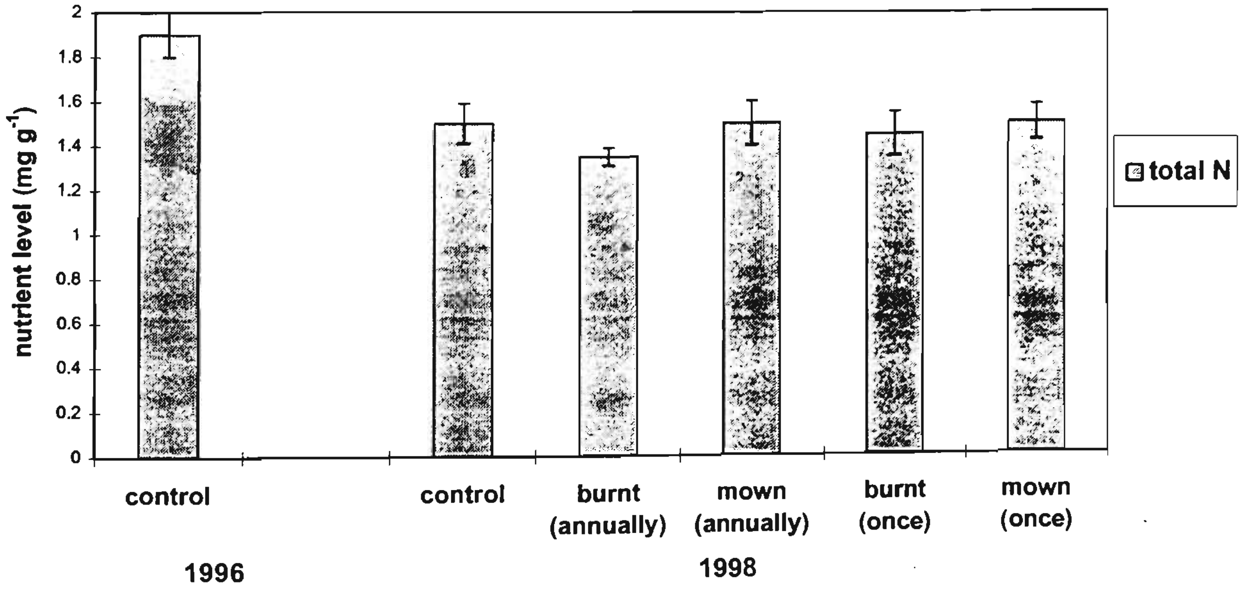

Fig. 8.6. Total levels of N in control plots (1996) and control, burnt (annually burnt and burnt once) and mown (annually and burnt once) plots (1998) at Laverton grassland reserve. Bars indicate standard errors (n = 8).

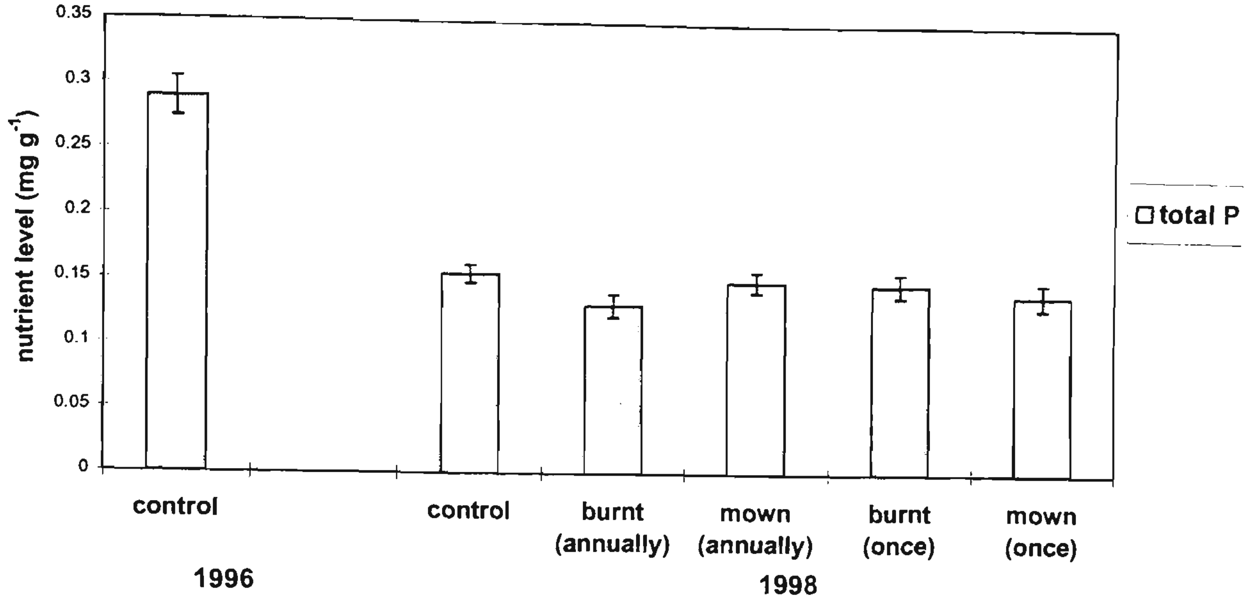

Fig. 8.7. Total levels of P in control plots (1996) and control, burnt (annually burnt and burnt once) and mown (annually and burnt once) plots (1998) at Derrimut grassland reserve. Bars indicate standard errors (n = 8).

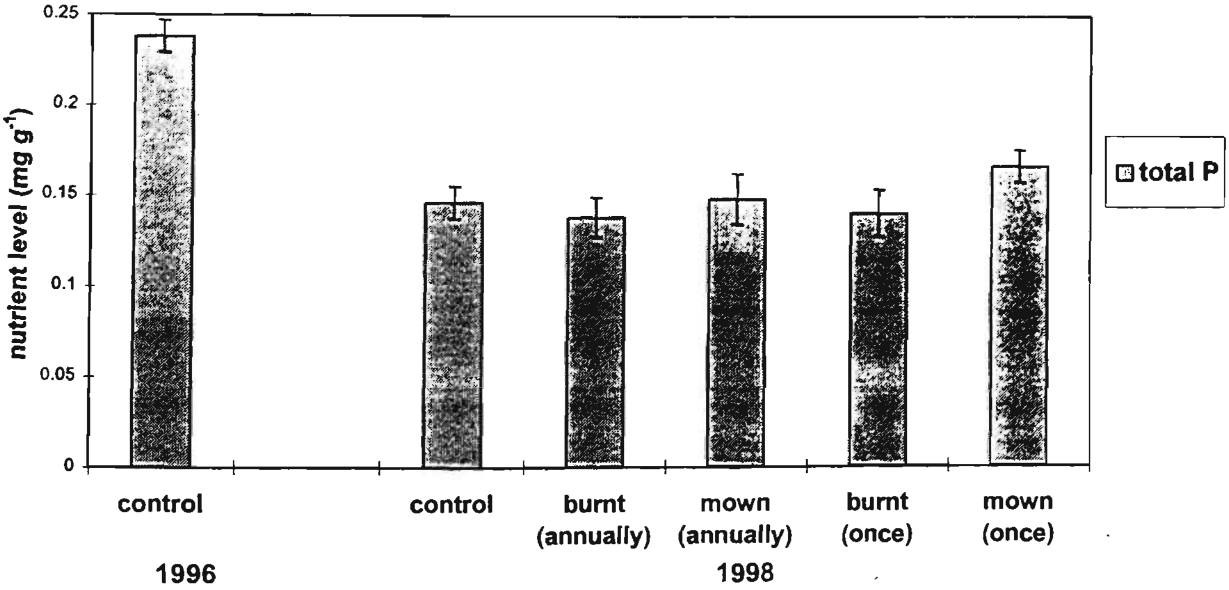

Fig. 8.8. Total levels of P in control plots (1996) and control, burnt (annually burnt and burnt once) and mown (annually and burnt once) plots (1998) at Laverton grassland reserve. Bars indicate standard errors (n = 8).

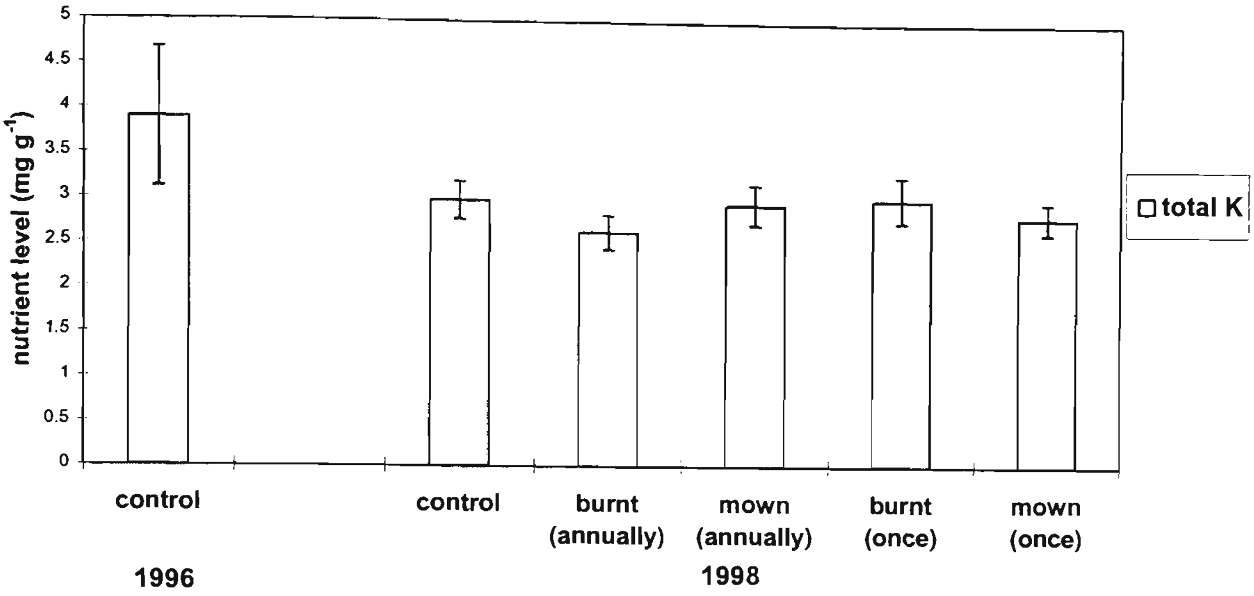

Fig. 8.9. Total levels of K in control plots (1996) and control, burnt (annually burnt and burnt once) and mown (annually and burnt once) plots (1998) at Derrimut grassland reserve. Bars indicate standard errors (n = 8).

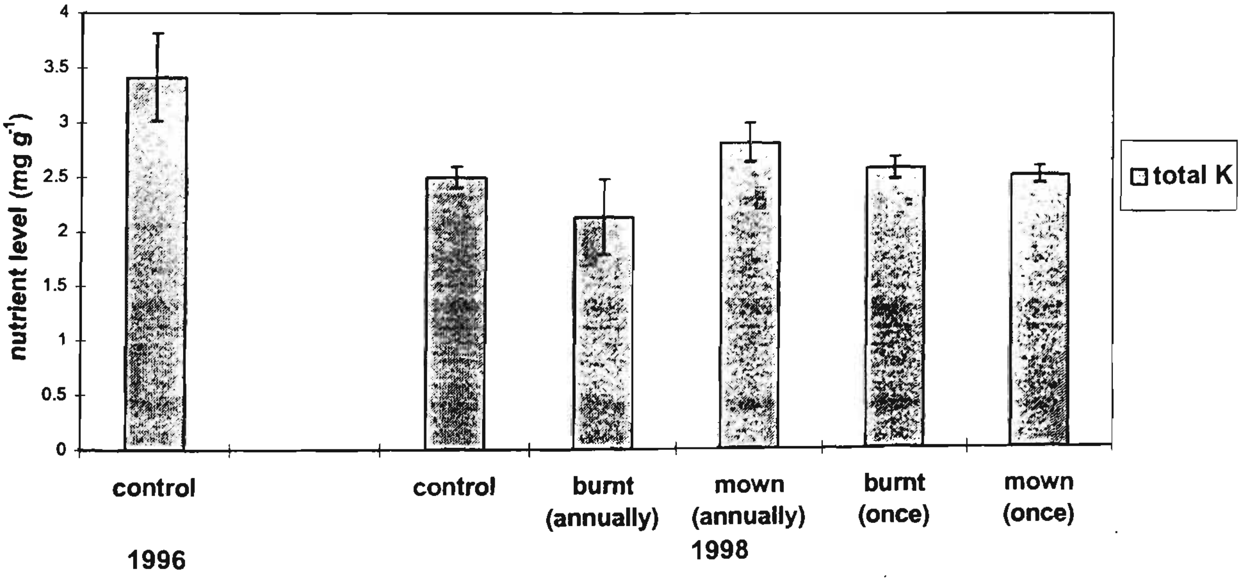

Fig. 8.10. Total levels of K in control plots (1996) and control, burnt (annually burnt and burnt once) and mown (annually and burnt once) plots (1998) at Laverton grassland reserve. Bars indicate standard errors (n = 8).

The total extractable nitrogen in annually burnt plots was lower than in unburnt (control) plots until one year after burning at Derrimut grassland reserve (Fig. 8.11). The total extractable nitrogen in annually burnt plots then increased markedly for spring 1997 and remained high thereafter. At Laverton grassland reserve, the spike in total extractable nitrogen occurred in the first summer following burning (February 1997), fell markedly in autumn 1997 and then reappeared in summer - autumn 1998 (Fig. 8.12). There were similar seasonal patterns of total extractable nitrogen (N) observed in control and in annually mown plots at both Derrimut and Laverton grassland reserves (Fig. 8.11 and Fig. 8.12). There was a tendency for total extractable nitrogen to be lower in summer than in spring and in autumn, at each of the trial sites. There was also an overall upward trend in total extractable nitrogen over the time that samples were taken in control and mown plots.

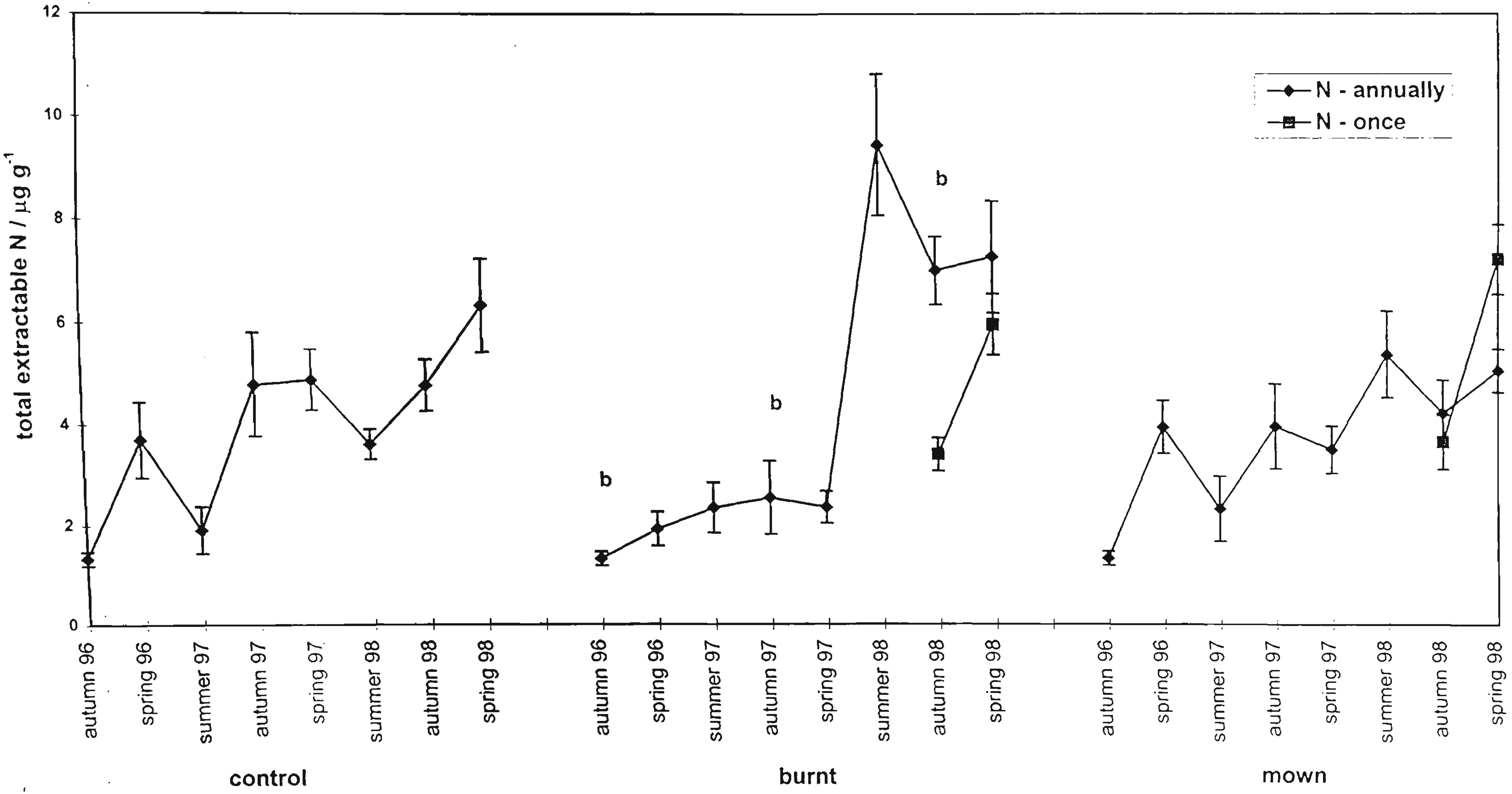

Fig. 8.11. Seasonal variation of available nitrogen in control, burnt (annually and once) and mown (annually and once) at Derrimut grassland reserve. (b = autumn burning took place). bars indicate standard errors (n = 8).

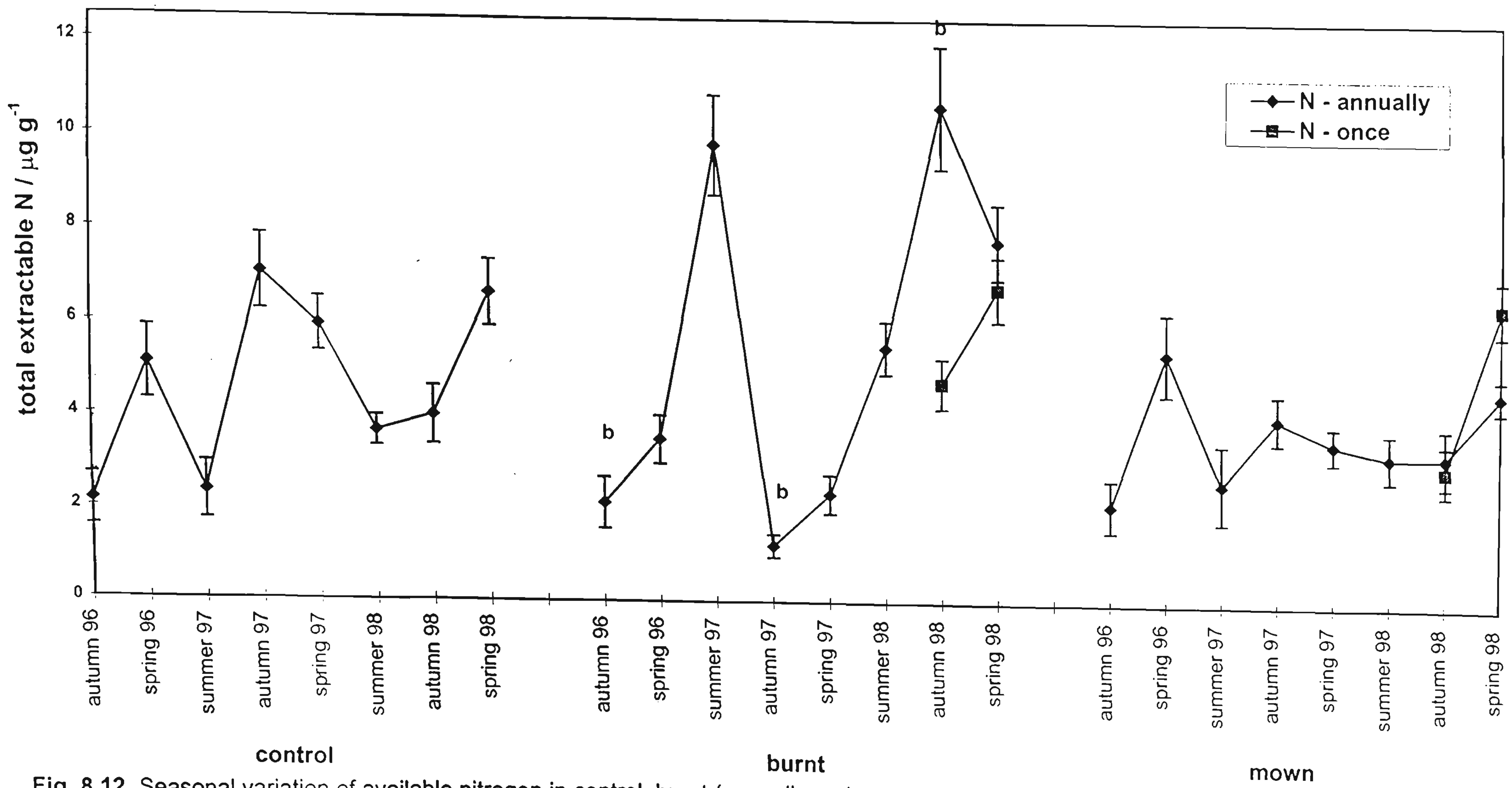

Fig. 8.12. Seasonal variation of available nitrogen in control, burnt (annually and once) and mown (annually and once) plots at Laverton grassland reserve. (b = autumn burning took place). Bars indicate standard errors (n = 8).

Fig 8.13 and Fig 8.14 show the seasonal variations extractable P in different seasons at Derrimut grassland reserve and Laverton grassland reserve respectively. Similar changes in the seasonal pattern of extractable phosphorus (P) were observed in control, annually burnt and annually mown plots. However the seasonal patterns were initially different at the two sites, Derrimut and Laverton grassland reserves. The extractable phosphorus (P) in the first year peaked in spring for all treatments at Derrimut grassland reserve whereas at Laverton grassland reserve the peak in the first year was in summer. After summer 1997 similar patterns were observed at the two sites. Two years after burning the extractable phosphorus (P) in burnt plots was significantly lower than in control plots ($p < 0.005$). Results were similar for each of the two grassland sites, Derrimut and Laverton grassland reserves.

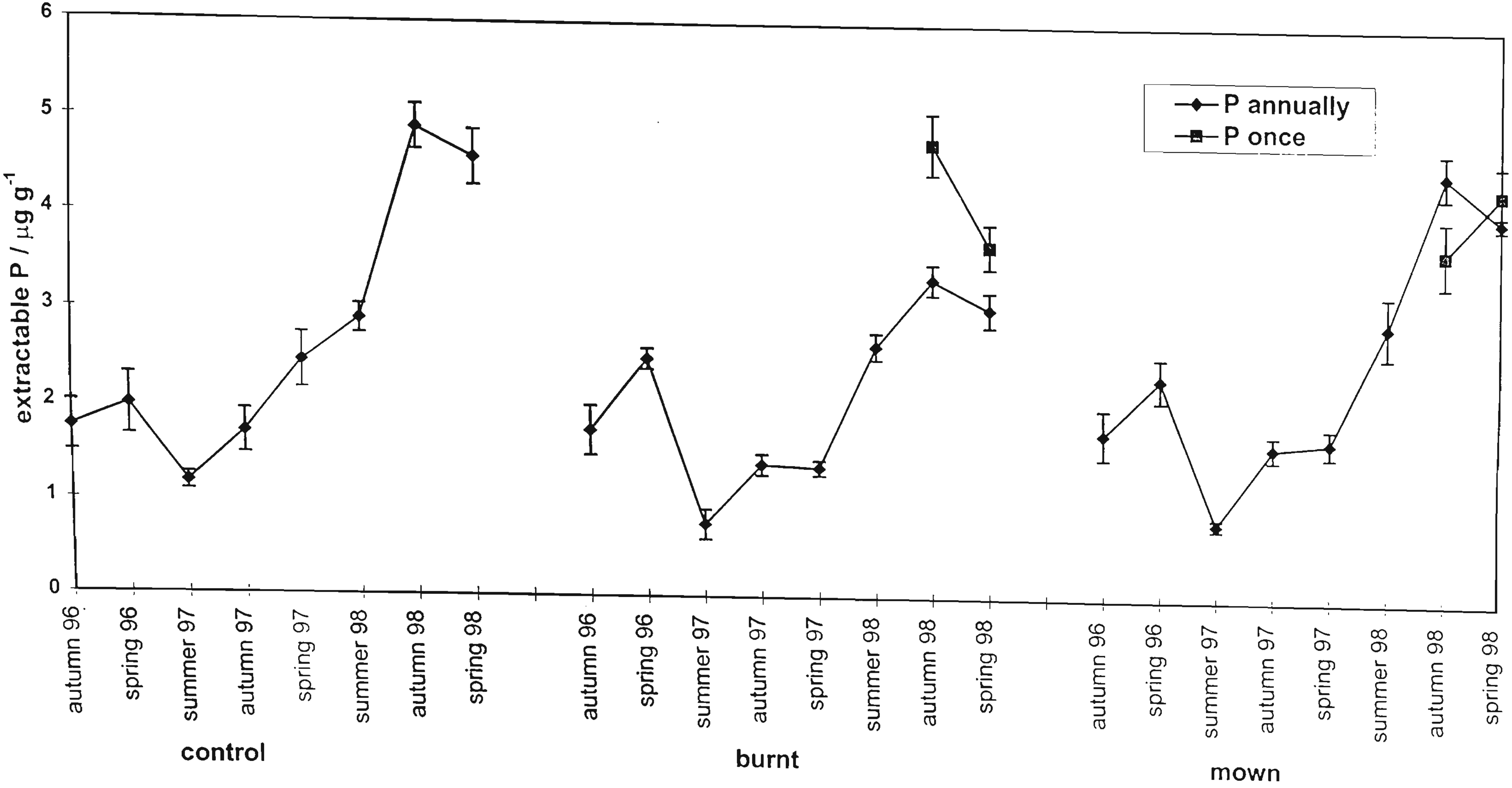

Fig. 8.13. Seasonal variation of extractable phosphorus (P) in control, burnt (annually and once) and mown (annually and once) plots at Derrimut grassland reserve. Bars indicate standard error (n = 8).

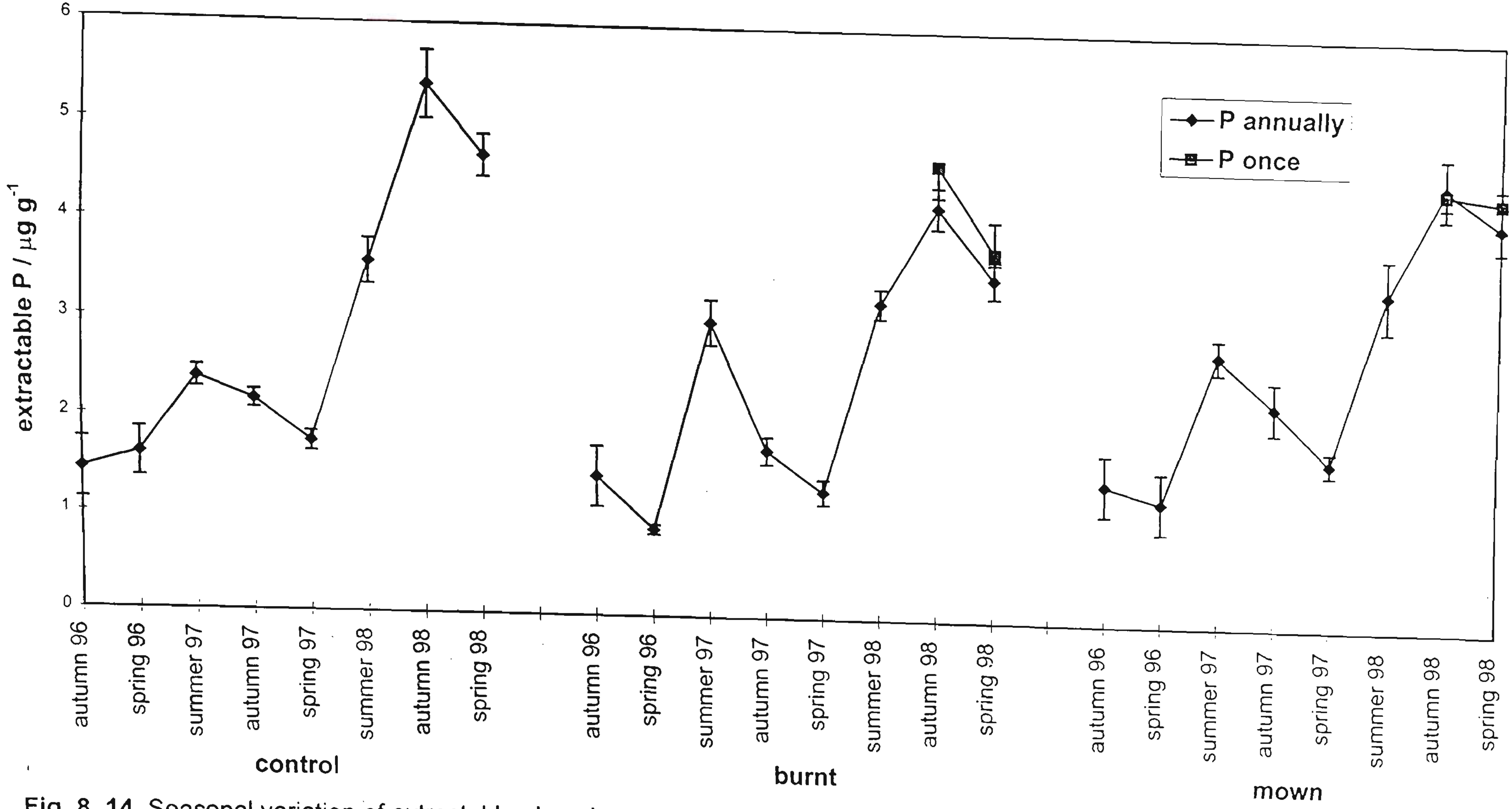

Fig. 8. 14. Seasonal variation of extractable phosphorus (P) in control, burnt (annually and once) and mown (annually and once) plots at Laverton grassland reserve. Bars indicate standard errors (n = 8).

The levels of extractable K in the soil in control (unburnt), burnt (annually and burnt once) and mown (annually and mown once) treatments are shown in Fig. 8.15 and Fig. 8. 16 at Derrimut grassland reserve and Laverton grassland reserve respectively. Between the sampling period of autumn 1996 to spring 1998 clearly showed that there was a similar seasonal pattern of extractable potassium (K) in the soil in each of the three treatments, control, annually burnt and annually mown plots (Fig. 8.15, Fig. 8.16). However, the seasonal patterns for extractable K at Derrimut reserve exhibited minor differences from patterns at Laverton reserve. The extractable potassium (K) in burnt plots in spring 1996 is significantly lower ($p < 0.01$) than in control plots. Thereafter, the extractable potassium (K) in burnt plots in spring was always significantly higher ($p < 0.01$) than in control plots every year until 1998. The same change was observed at both grassland sites, Derrimut and Laverton grassland reserves.

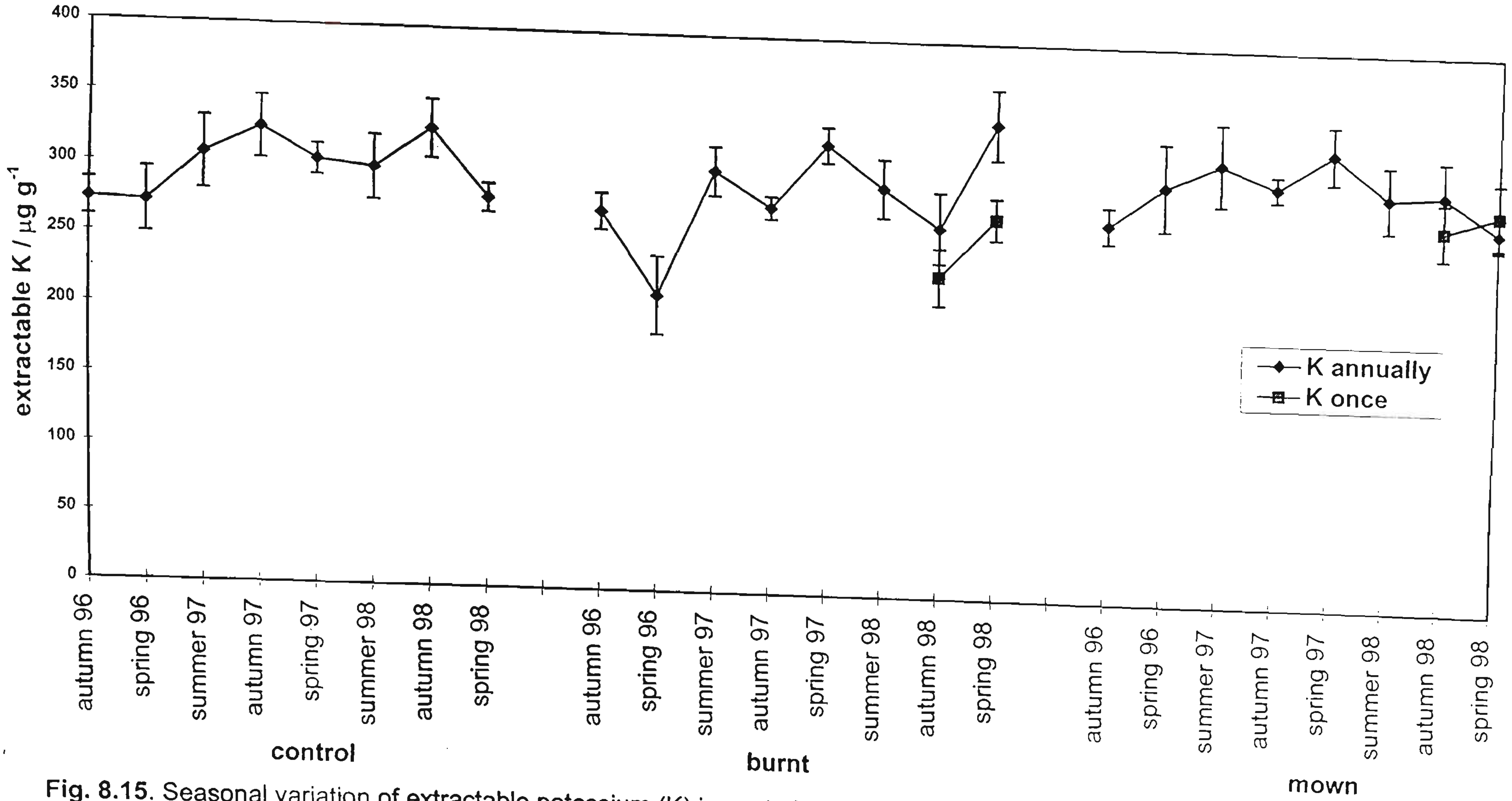

Fig. 8.15. Seasonal variation of extractable potassium (K) in control, burnt (annually and once) and mown (annually and once) plots at Derrimut grassland reserve. Bars indicate standard errors (n = 8).

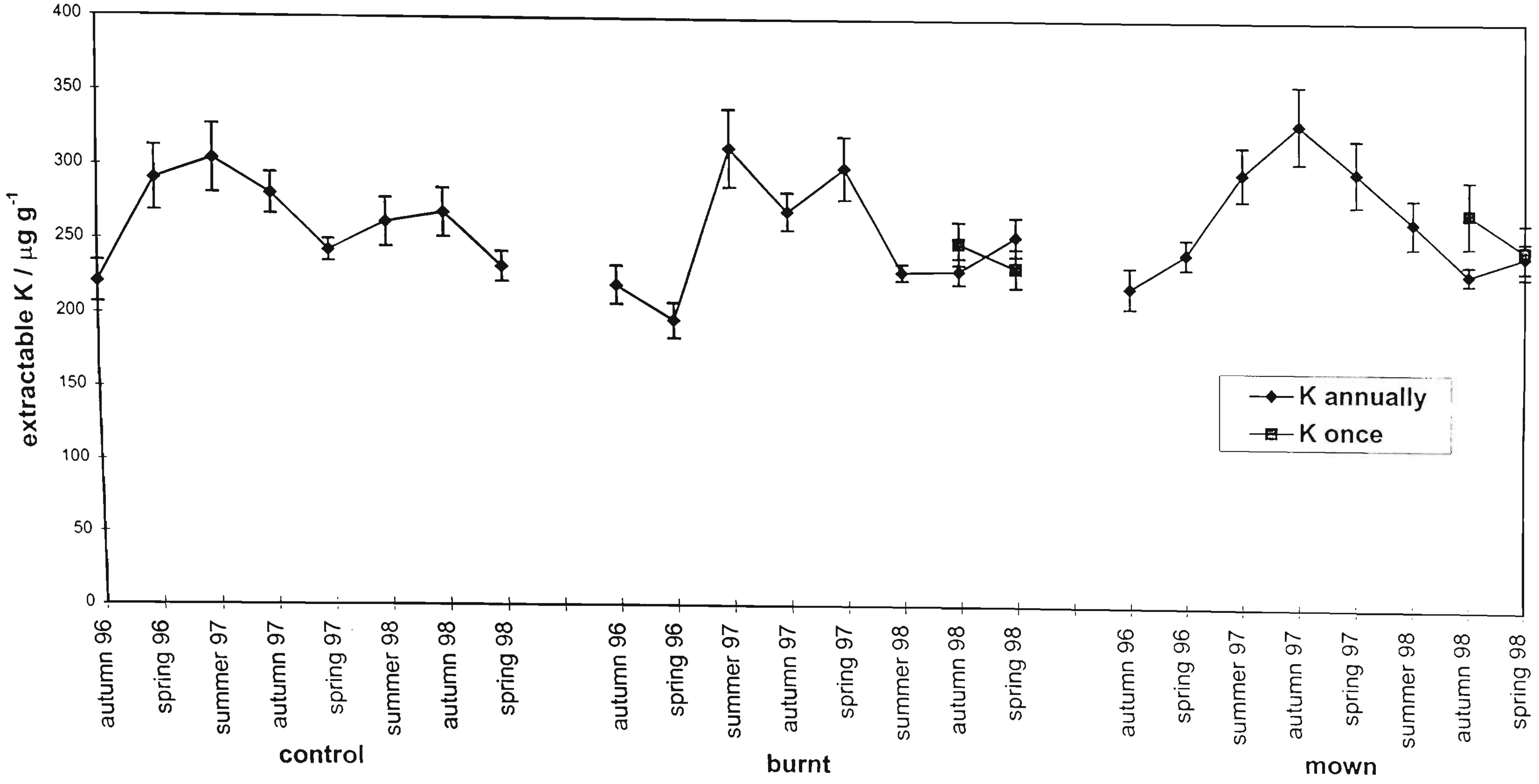

Fig. 8. 16. Seasonal variation of extractable potassium (K) in control, burnt (annually and once) and mown (annually and once) plots at Laverton grassland reserve. Bars indicate standard errors (n = 8).

In figures (Fig. 8.17 to Fig. 8.25) the changes between years in each season of extractable nutrients (N,P & K) are shown for control, burnt and mown plots over the sampling period 1996 to 1998 at Derrimut and Laverton grassland reserves.

These results demonstrate the extent to which extractable nitrogen (N), phosphorus (P) and potassium (K) are similar across treatments and sites, for each season.

There were similar patterns of extractable nitrogen (N) in autumn across two sites Derrimut reserve and Laverton reserve in each of three treatments (Fig. 8.17). There were also similar patterns of extractable nitrogen across control and mown treatments.

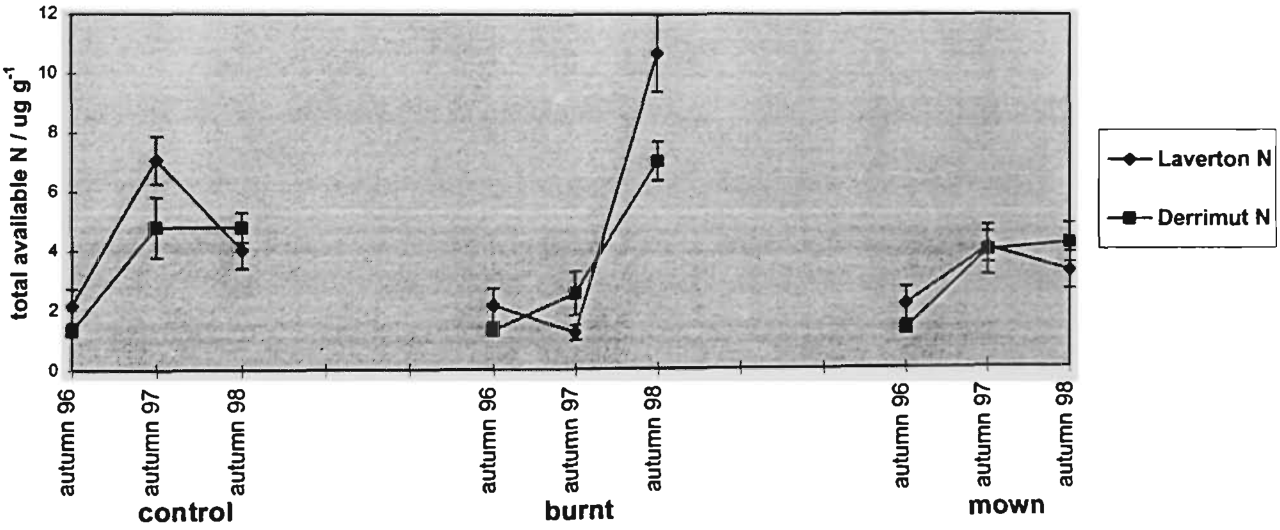

Fig. 8. 17. Extractable N in control, burnt and mown plots in autumn 1996 to 1998 at Derrimut and Laverton grassland reserves. Bars indicate standard errors (n = 8).

There were similar patterns of extractable nitrogen (N) in spring (1996 -1998) across two experimental sites Derrimut and Laverton grassland reserves. The patterns across treatments were different (Fig. 8.18).

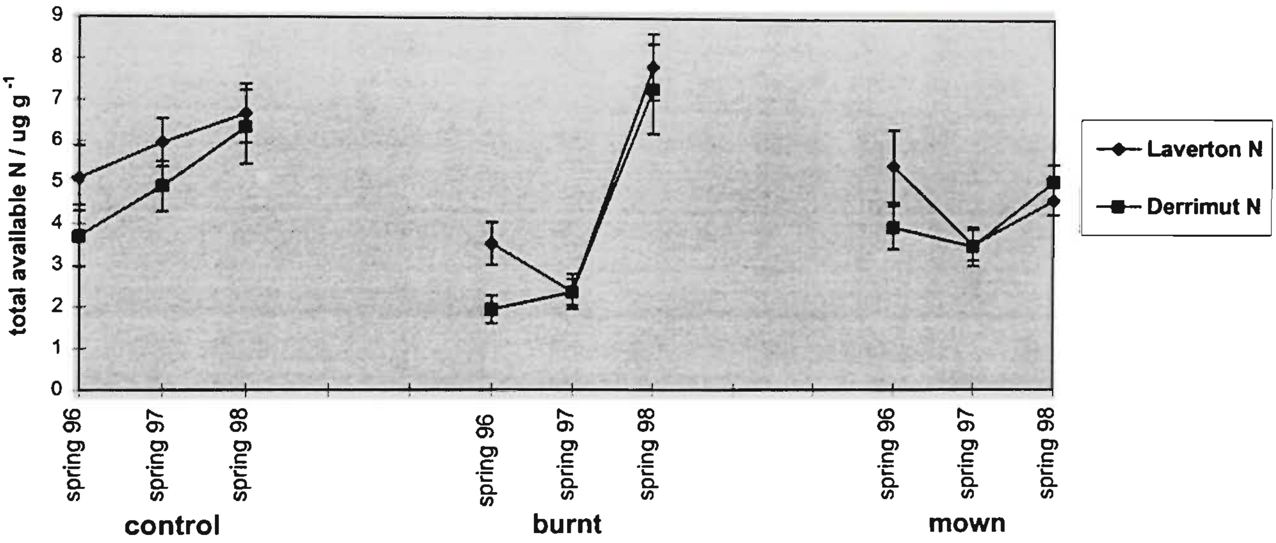

Fig. 8. 18. Extractable N in control, burnt and mown plots in spring 1996 to 1998 at Derrimut and Laverton grassland reserves. Bars indicate standard errors (n = 8).

The extractable nitrogen (N) in summer exhibited different patterns in burnt plots at two sites Derrimut and Laverton grassland reserves (Fig. 8.19). There were similar patterns of extractable N across control and mown plots and at two sites.

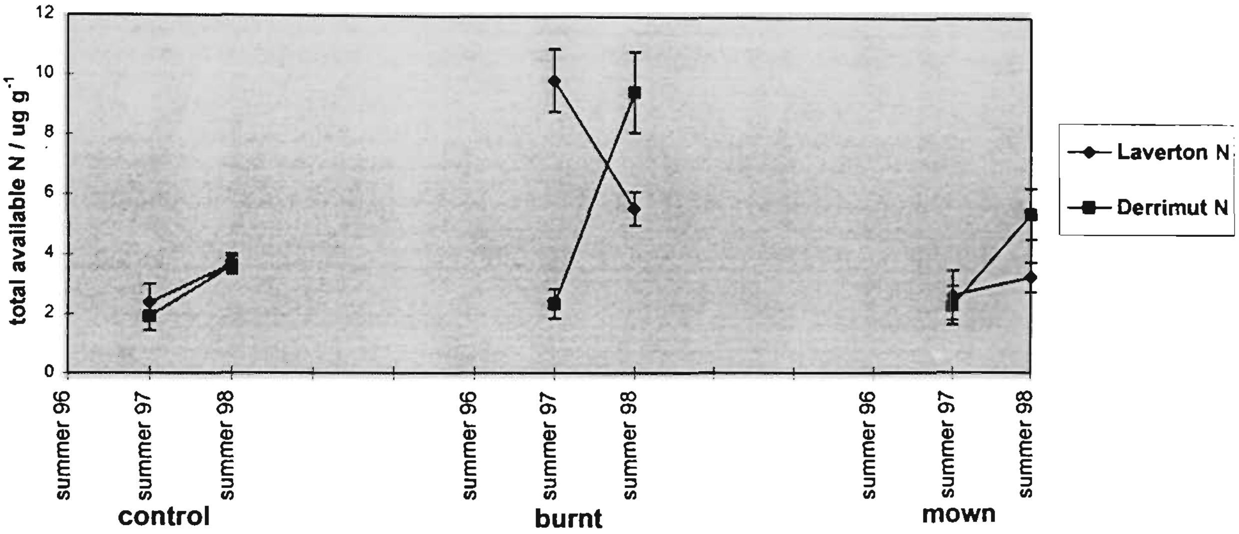

Fig. 8. 19. Extractable N in control, burnt and mown plots in summer 1996 to 1998 at Derrimut and Laverton grassland reserves. Bars indicate standard errors (n = 8).

There were similar patterns of extractable phosphorus (P) in autumn (1996 to 1998) across treatments and across two experimental sites Derrimut and Laverton grassland reserves (Fig. 8.20).

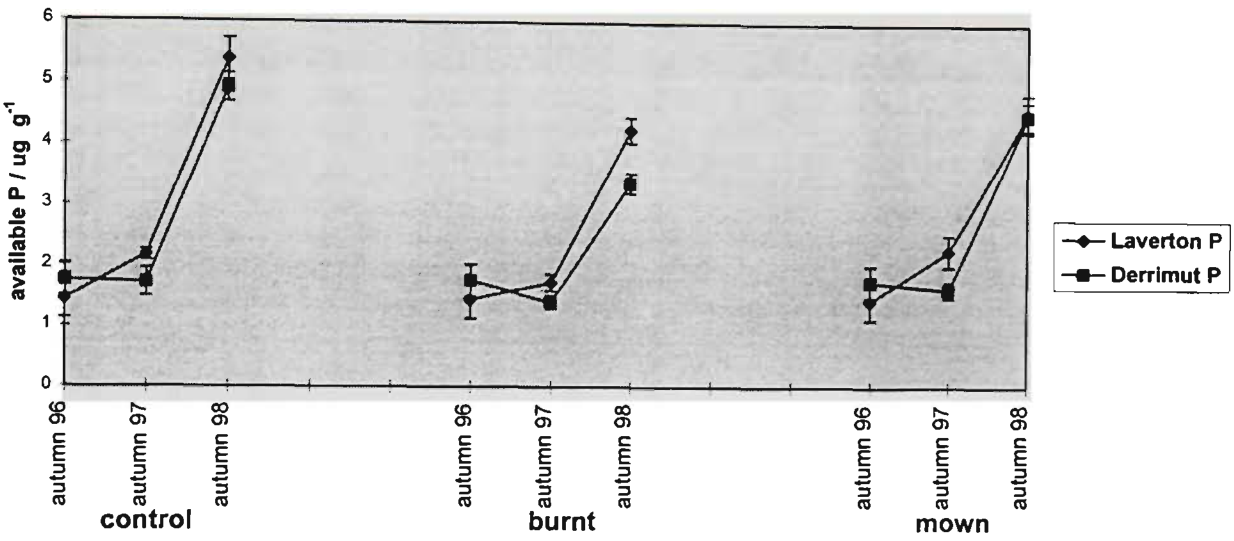

Fig. 8. 20. Extractable P in control, burnt and mown plots in autumn 1996 to 1998 at Derrimut and Laverton grassland reserves. Bars indicate standard errors (n = 8).

The extractable phosphorus (P) in spring (1996 to 1998) exhibited similar patterns in control plots across two sites. The patterns of extractable phosphorus in burnt and mown plots across two sites were different (Fig. 8.21).

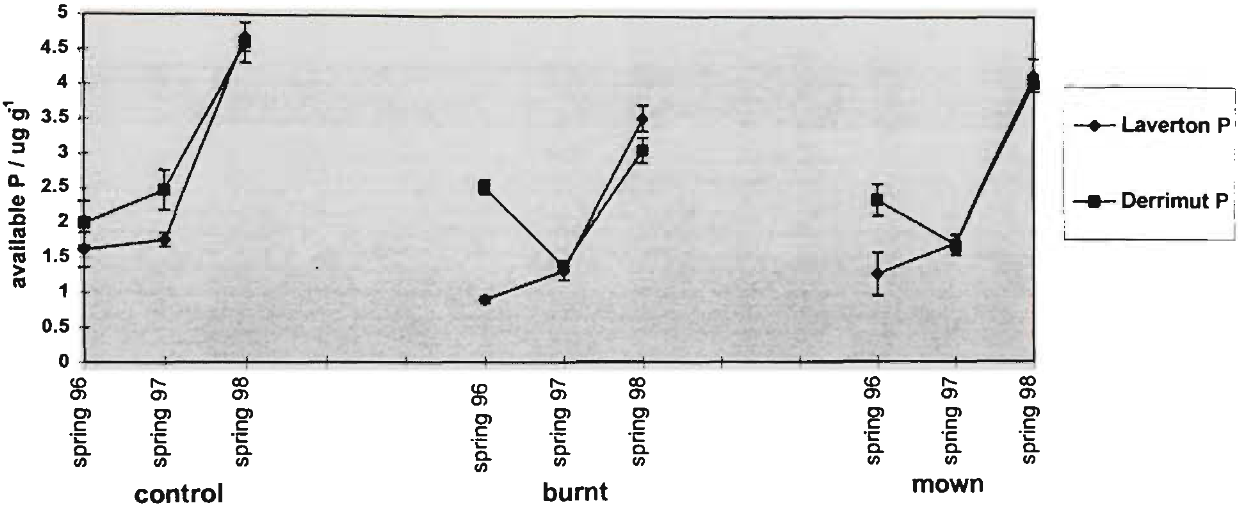

Fig. 8. 21. Extractable P in control, burnt and mown plots in spring 1996 to 1998 at Derrimut and Laverton grassland reserves. Bars indicate standard errors (n = 8).

There were similar patterns of extractable phosphorus (P) in summer across sites and across treatments (Fig. 8.22).

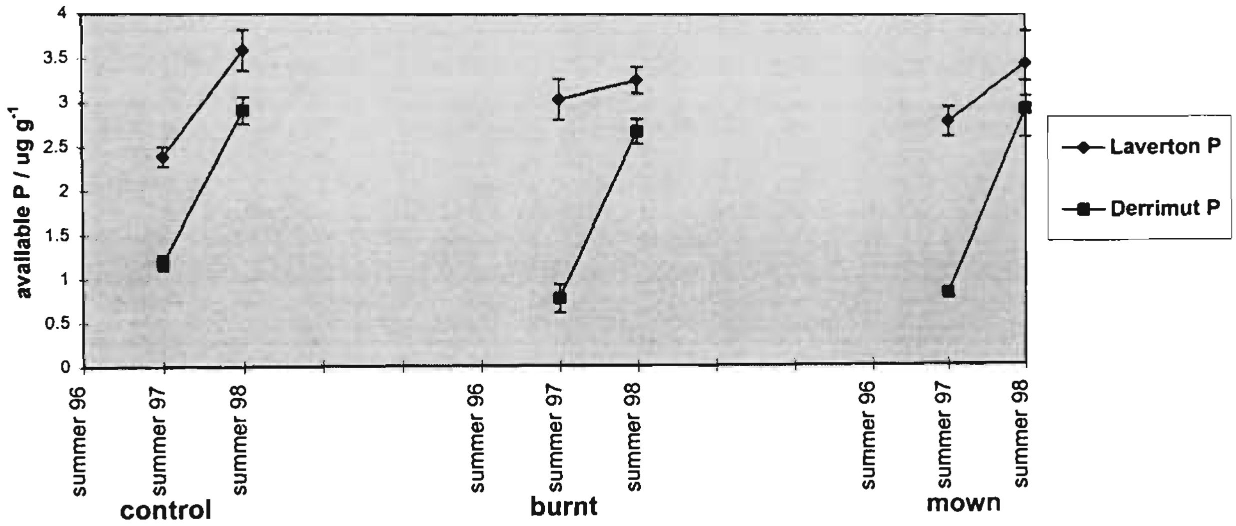

Fig. 8. 22. Extractable P in control, burnt and mown plots in summer 1996 to 1998 at Derrimut and Laverton grassland reserves. Bars indicate standard errors (n = 8).

The extractable potassium (K) in autumn exhibited similar patterns across control and burnt plots (Fig. 8.23). The patterns were similar across two sites.

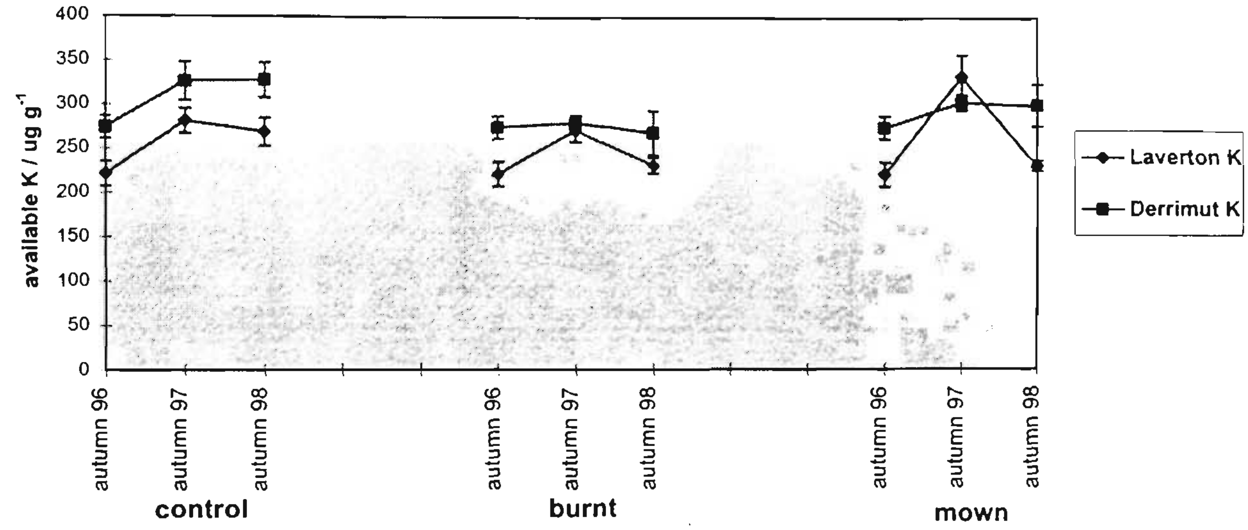

Fig. 8. 23. Extractable K in control, burnt and mown plots in autumn 1996 to 1998 at Derrimut and Laverton grassland reserves. Bars indicate standard errors (n = 8).

The extractable potassium (K) in summer exhibited similar patterns across two sites in burnt and mown plots. There were general similar patterns across burnt and mown treatments. The patterns were different in control plots at two sites (Fig. 8.24).

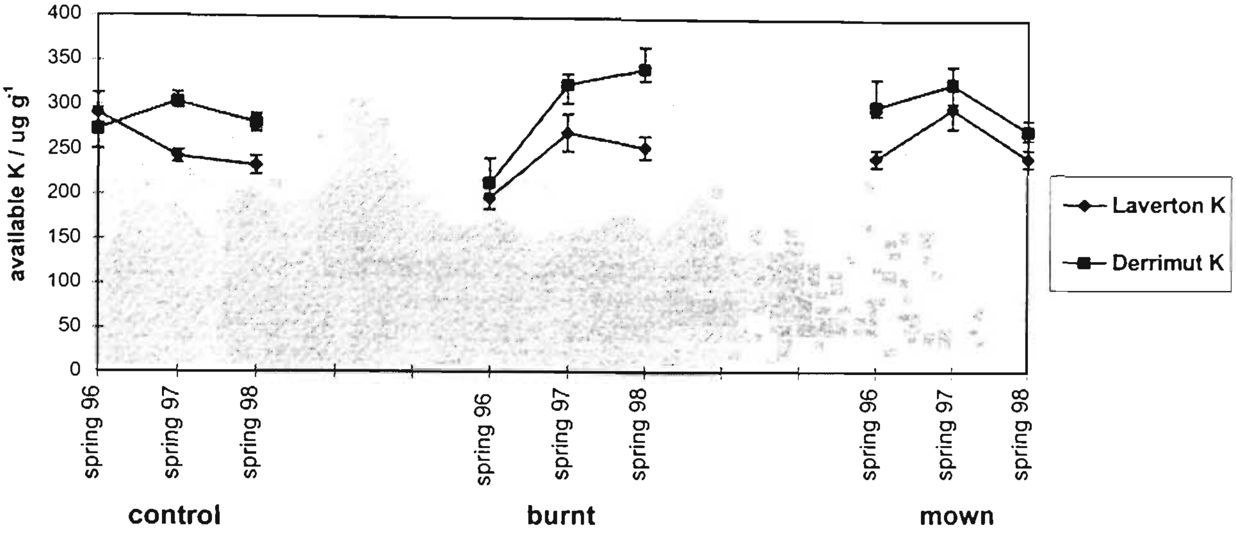

Fig. 8. 24. Extractable K in control, burnt and mown plots in spring 1996 to 1998 at Derrimut and Laverton grassland reserves. Bars indicate standard errors (n = 8).

The were similar patterns of extractable K in summer (1996 - 1998) across treatments and across two sites (Fig. 8. 25) Derrimut and Laverton grassland reserves.

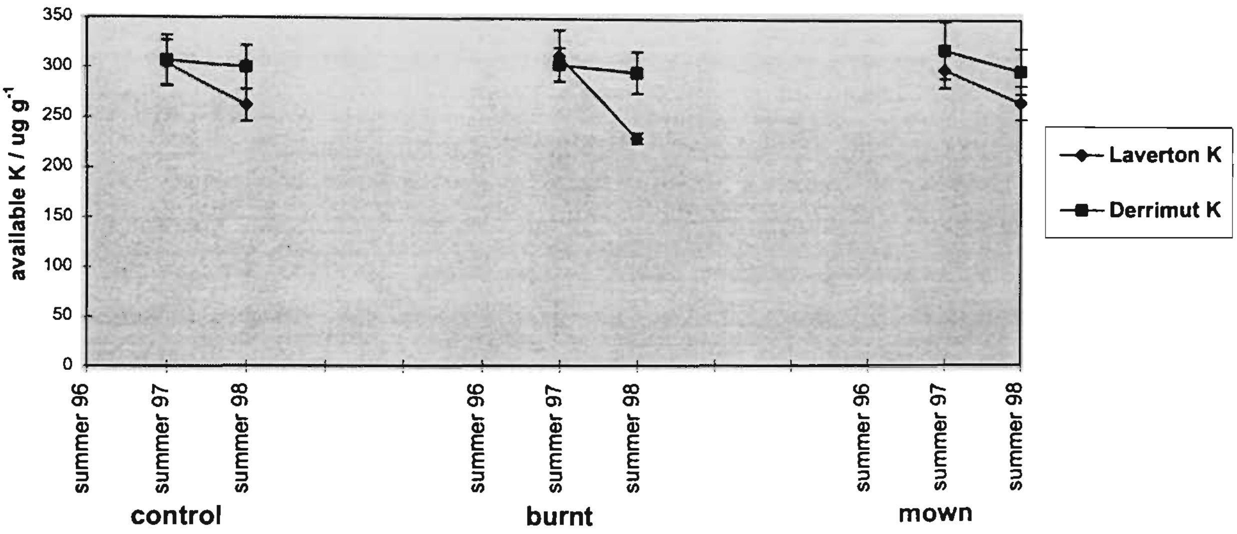

Fig. 8. 25. Extractable K in control, burnt and mown plots in summer 1996 to 1998 at Derrimut and Laverton grassland reserves. Bars indicate standard errors (n = 8).

Initial and final soil pH in control plots and each of the treatment (annually burnt, annually mown, burnt once and mown once) plots are described in Table 8.1 and Table 8.2 for Derrimut and Laverton grassland reserves respectively. Soil pH is significantly higher ($p < 0.01$ both grasslands) in annually burnt plots than in control plots. Soil pH in annually mown plots were not significantly different ($p > 0.1$ both grasslands) to control plots.

Table 8. 1: Soil pH (initial and final) in control, burnt and mown plots at Derrimut grassland reserve.

Time	control	ann. burnt	ann. mown	burnt once	mown once
initial (autumn 96)	6.15 (0.07)	6.15 (0.07)	6.15 (0.07)	6.15 (0.07)	6.15 (0.07)
final (spring 98)	6.18 (0.07)	6.42 (0.08)	6.30 (0.05)	6.31 (0.07)	6.20 (0.08)

Mean values of pH are given. Standard errors are in brackets ($n = 8$).

Table 8. 2: Soil pH (initial and final) in control, burnt and mown plots at Laverton grassland reserve.

Time	control	ann. burnt	ann. mown	burnt once	mown once
initial (autumn 96)	6.00 (0.07)	6.00 (0.07)	6.00 (0.07)	6.00 (0.07)	6.00 (0.07)
final (spring 98)	6.04 (0.05)	6.22 (0.03)	6.14 (0.05)	6.27 (0.04)	6.05 (0.04)

Mean values of pH are given. Standard errors are in brackets ($n = 8$).

8.4 Discussion

8.4.1 Soil pH

Two years after burning, soil pH in burnt plots was significantly higher ($p < 0.01$) than in control plots (Table 8.1 and Table 8.2). Cook (1939) found the similar variation of pH across burnt and unburnt grasslands. He found that annual burning of *Themeda* dominated grasslands in South Africa raised the pH from 5.8 to 6.7 in the upper 38 mm of soil. According to Moore (1960) the pH of Nigerian savanna soil was raised by annual burning from 6.0 to 6.2, if the burns were late in the dry season and very hot, or to PH 6.3 if the burning was early in the dry season, when temperatures were not so high. Ehrenreich and Aikman (1963) showed that grassland fires in Iowa increased the pH of the upper 18 mm of the soil from 5.8 to 6.1. Daubenmire (1968) pointed out that the changes of pH in burnt plots persisted for only a year or two.

8.4.2 Available nutrients

Overall the levels of available nutrients were markedly variable across seasons. Different seasonal patterns of available nutrients were also observed between treatments. In addition, the seasonal patterns were different for different nutrients. However, despite these variations, there were remarkable similarities in patterns of extractable nutrients between Derrimut and Laverton grassland sites in some seasons for some treatments. This suggests that similar factors may have been operating at the two sites to determine the overall levels of extractable N, P and K. As a general trend, the available levels of nitrogen (N) and phosphorus (P) increased over three years (from 1996 to 1998) across most seasons. After three years the available nitrogen (N) in burnt plots was significantly higher ($p < 0.01$) than control (unburnt) and mown plots in all seasons except spring. This trend was observed at both sites. The available phosphorus (P) was significantly lower ($p < 0.01$) in annually burnt plots than control and mown plots in autumn and spring after three years but similar levels of P was found in summer across all treatments.

8.4.2.1 Available Phosphorus (P)

Overall there was remarkable similarity in both the levels and seasonal patterns of extractable phosphorus between Derrimut and Laverton North grasslands (Figures 8.13 and 8.14). This suggests that similar factors were operating across the two sites to determine the levels of available phosphorus. The reasons for the overall increase in extractable P over the three years of monitoring at each of the sites across all treatments are unknown, but may be due to the extended drought period that affected all sites over the second two years of the trials. Drought may have lead to death of either microbes in the soil, or the fine root hairs of native species, or both, which would in turn have led to an increase in available P, especially if the growth of other species on site, such as native forbs and exotic annual grasses and flatweeds, was also limited by the drought conditions, preventing large-scale uptake of available P by these species. A similar general trend of increasing extractable nitrogen across sites and treatments was also observed (see section 8.4.2.3 below), in accord with the suggestion that plant or microbial death was involved.

Two years after burning the lower available phosphorus (P) in burnt plots compared to control plots, is possibly attributable to the different soil pH values in burnt and unburnt (control) plots. The soil pH in burnt plots was significantly higher ($p < 0.01$) than in control plots (see above). Moore (1960) found that mild fires coming at the start of the dry season reduced available phosphorus (P) in Nigerian savanna, but hot fires coming late in the dry season increased available phosphorus. The available phosphorus (P) in burnt once plots was also significantly lower ($p < 0.01$) than in unburnt (control) plots. This is probably due to higher soil pH in burnt once soil than in control plots. The available phosphorus in the current study was not significantly different across annually burnt and burnt once treatments at either site, Derrimut and Laverton grassland reserves (Figures 8.13 and 8.14). There was no significant different of available phosphorus (P) observed between control and annually mown plots in any season. This was same at each of the sites, Derrimut and Laverton. It would appear that mowing has little effect on levels of available phosphorus, compared with controls.

8.4.2.2 Available potassium (K)

In general, there was remarkably little variation in the levels of extractable potassium (K) across treatments, sites and seasons (Figures 8.15 and 8.16). There was a tendency for extractable K levels to increase in the summer and autumn seasons in control plots, by about 10 percent. The reasons for this are unknown. The available potassium (K) in burnt plots in spring decreased significantly after the first burn in autumn 1996, compared with control and mown treatments. The same change was observed at each of the grasslands, Derrimut and Laverton grasslands. This might be because of leaching of potassium (K) from the soil, as soil was very moist and soft at this time and increased filtration of water was likely with the complete removal of above-ground vegetation by the fire.

Thereafter, the available potassium (K) in burnt plots in spring was always significantly higher than in control plots every year until 1998. The reason for this may have been because of less leaching losses through the soil in burnt plots because soil had become harder and comparatively less soil moisture content than in control plots after several repeated burns. Therefore there would have been less leaching losses of potassium (K) in burnt plots and combustible products of plant materials containing potassium would have been retained in the soil. An alternative explanation for the higher K levels in burnt treatments in spring, compared with control and mown treatments, is that the drought conditions may have affected plant growth, and possibly promoted death of plant roots and soil microbes in ways that lead to increases in available K. The levels of available potassium (K) in burnt plots decreased in summer and autumn. The same trend was observed at each of the grassland sites, Derrimut and Laverton grassland reserves. Similar increases in K in burnt plots compared with control plots were obtained by Trabaud (1983 b & 1994) who found that available potassium (K) in the soil increased after fire but there was no difference in available potassium between burnt and unburnt treatments after two years in Mediterranean type ecosystems. There was no consistent seasonal pattern of available potassium (K) in annually mown plots between the two experimental sites, Derrimut and Laverton grassland reserves or between control plots and annually mown plots after two years. It was also observed that the available potassium (K) in burnt once plots was significantly lower than in annually burnt plots after two years. Results were the same at each of the grassland reserves, Derrimut and Laverton. There was no significant difference in available potassium (K) between annually mown and mown once treatments after three years.

8.4.2.3 Available nitrogen (N)

Of the three extractable nutrients monitored (N, P and K), nitrogen was the most affected by the treatments applied (Figures 8.11 and 8.12). While extractable nitrogen (N) levels in control and mown plots followed broadly similar seasonal patterns, both within sites and across sites, extractable N levels in burnt plots were markedly different from the other two treatments at particular times of the year, and also exhibited some differences at particular times between sites. The available nitrogen in burnt plots at Derrimut grassland reserve was lower than in unburnt (control) plots in the first year following burning. The results were similar at Laverton grassland reserve for spring and autumn, but exhibited a remarkable surge in extractable N levels over the first summer following burning. The total extractable nitrogen in annually burnt plots exhibited a second marked surge in the summer of 1998 plots at both Derrimut and Laverton North grassland reserves (Fig. 8.11 and Fig. 8.12).

There are several feasible explanations for these summer surges in total extractable nitrogen following previously low levels in the spring. Several studies of grasslands and related communities (Woodmansee & Wallach 1981; Arianoutson & Margaris 1982; Raison 1979) have shown that soil bacterial numbers built up rapidly after fire. According to this model the higher microbial counts in burnt plots would have immobilised nitrogen (N) and decreased available nitrogen in the soil in the spring (and throughout spring, summer and autumn of the first year at Derrimut grassland reserve). Ojima (1987) pointed out that annually burnt sites have the highest microbial immobilisation of nitrogen and phosphorus. This is because annual burning decreased carbon (C) content in the soil because the carbon (C) from dead roots produced as a result of the fire is consumed by rapidly increasing numbers of soil micro- organisms. A similar process of nutrient immobilisation occurs when an available carbon source, such as sugar, is added to the soil, as were seen in the trials reported in chapters 5 and 7. The organic carbon (C) content at this period in soil under annually burnt plots was found to be consistently less than in control plots or mown plots (Figures 8.3 and 8.4). Similar results were obtained by Ojima et al. (1994) who found that repeated annual burning of tallgrass prairie decreased soil organic matter. When the organic carbon resources in the soil becomes too low to maintain the growth of populations of soil micro-organisms, massive microbial death occurs and the immobilised nitrogen (N) is released back to the soil in a surge, to be taken up again by plants (natives and weeds) when suitable conditions become available for plant growth in the autumn.

Another possible explanation for the high nitrogen (N) level in burnt plots compared to control plots may be related to changes in nitrogen fixation by soil-borne bacteria. Debano & Conrad (1978) found that nitrogen fixing bacteria increased after fire and increased available nitrogen content in the soil. Meiklejohn (1955) studied the microflora of the top 25 mm of soil following burning experiments in grasslands in Kenya. She explained that anaerobic nitrogen fixers (*Clostridium* sp.) persisted through the burning, and the total nitrogen (N) content in the soil increased due to the comparatively higher destruction by fire of nitrifying bacteria than of nitrogen fixing micro organisms. The high relative levels of ammonium in the soil observed at both

Derrimut and Laverton grassland reserves at the end of winter, when waterlogged soils would have prevented conversions to nitrate (data not shown), is evidence of high nitrogen fixation rates at these sites.

There is a third possible explanation for the low spring levels of extractable N followed by extreme surges in summer and low levels again in autumn, which relates to the death of some plant parts over the extreme summer droughts experienced in both years that data was collected. According to this explanation, efficient removal of above-ground biomass by fire can stimulate the growth of otherwise senescent kangaroo grass. Evidence for this effect can be seen in the relatively low levels of total extractable nitrogen in burn plots in spring, and in mown plots at various times during the year, compared with control plots (Figures 8.11 and 8.12). However, burning also causes high levels of stress on plants subjected to drought conditions, and over the summer there is some death of plant material, as evidenced by reduced total carbon levels in the soils under burnt plots, compared with control and mown plots (Figures 8.3 and 8.4). Plant death leads to release of nutrients in the soil over summer, when there is little growth of either other plants or soil microbes, so a surge in extractable nitrogen is recorded. In autumn and spring, when more water is available and plant growth (native and exotic) is higher, there is rapid uptake of the nitrogen released to the soil, and extractable nitrogen levels are observed to fall rapidly.

None of the three models outlined above, which attempt to explain the extreme oscillations in total extractable nitrogen between spring, summer and autumn, act in contrary ways to the other models – each model would produce approximately the same result. Some may even be complementary. For example, the effects on extractable soil nitrogen of plant death in the autumn following burning could be masked to some extent by uptake of nitrogen by soil microbial growth, encouraged by the additional carbon in the soil from dead plant material. It is possible, and even likely, that the patterns of extractable nitrogen observed in burn treatments were a combination of all three processes.

Two years after burning the available nitrogen (N) in annually burnt plots reached similar levels to control (unburnt) plots. Results were similar at both Derrimut and Laverton grassland reserves.

The available nitrogen (N) in burnt once treatments showed different patterns to burnt annually treatments and there were also differences across the two sites. At both sites the available nitrogen (N) in burnt once treatments was significantly lower than in annually burnt plots when measured in the autumn and spring of 1998. The available nitrogen (N) reached similar levels in controls, annually burnt and burnt once, at two years after burning. The results were same at both sites, Derrimut and Laverton grassland reserves. These results are consistent with the proposal that burning causes plant death and release of available nitrogen, because a single burn would be expected to have less effect on plant death, so surviving plant tissue, with overburden removed, might be expected to grow quickly, take up nitrogen and result in lower levels of available nitrogen in the soil than for treatment where ongoing burning and associated plant death would cause higher available soil nitrogen levels.

The similar seasonal pattern of extractable N between control and annually mown treatment suggests that annually mown treatment has no greater effect on available soil N in native grassland soils, other than to keep extractable N slightly lower than equivalent unmown treatments. The minor differences between mown and control plots are likely to be due to the increased growth of Kangaroo Grass on mown plots compared with control plots, prompted by the removal of standing dead biomass

8.4.3 The effects of available soil nutrient levels on the weed content of vegetation

According to Henderson and Hocking (1998), neither the average cover or biomass of annual grasses or broadleaf weeds was significantly different on quadrats which had been burnt or mown, annually or once. This was an unexpected result, in terms of the widely varying levels of extractable nitrogen in soils of annually burnt grasslands in particular (Figures 8.11 and 8.12), because results reported in Chapters 5 and 7 show that increasing soil nutrient levels lead to increases in weed biomass. The results suggest that there are key parameters influencing on the growth of weeds in intact native grassland remnants (i.e. remnants without soil disturbance) that are additional to the levels of available soil nutrients. Such factors could be the effect of fire on the soil seedbank of weeds, the effects of various management treatments on micchorizael growth of plant roots, and the effects of burning on surface crusts. Why levels of available nitrogen vary so widely in annually burnt grasslands, and why elevated available nitrogen levels do not influence the growth of annual grasses and broadleaf weeds in native grassland remnants, requires further investigation.

In a recent study by Matthews (1999) of the germination and growth characteristics of the annual grasses *Briza maxima* and *Vulpia bromoides* in the same plots examined for this study, the average biomass of plants was found to be almost double in annually burnt plots than in annually mown plots. However, the number of germinants of *Briza maxima*, in particular, were greatly reduced in burnt plots compared with mown plots. It is possible that the increase in biomass of annual weeds due to increased available nutrient levels in annually burnt plots could be offset by the reduced number of germinants due to the effects of burning on the soil seed bank of some annual grass species.

Chapter 9. Implication of Outcomes for Lowland Grassland Management.

In the recent past, managers of lowland native grasslands have focussed largely on the influences of above-ground competition for light, and the contents of the soil seedbank, to explain the levels and types of weediness in native grassland remnants. The outcomes of the investigations reported in this thesis clearly demonstrate that below-ground factors, and in particular levels of available nitrogen and phosphorus, have significant influences on the levels of weediness in western (basalt) plains grassland remnants.

Methods to minimise and manage weeds in disturbed in disturbed native grassland sites will need to take into account the very high levels of available N and P stored underground in plant parts and micro-organisms, which can be rapidly released as a result of plant death during soil disturbance, and promote rapid weed growth. One implication of this study is that native grassland vegetation would appear to be very effective in removing available N and P from the soil, which in turn acts to reduce the growth of annual grasses and flatweeds. Management practices which lead to increases in available N and P in the soil will most likely promote the growth of these weeds, largely by causing increases in the size of existing seedlings on a site, rather than increases in the number of seedlings.

Burning practices have significant effects on the levels of available N and P in the soil under plains grassland remnants, with potential influences on levels of weediness. However, the effects of fire on a range of other parameters which can affect weed levels in native grasslands (for example, seedbank, microbial growth, nitrogen fixation, plant growth affecting nutrient uptake) are not yet sufficiently well understood to apply burning across the board as a control measure for annual grasses and flatweeds.

Nevertheless, experience of grassland management to prevent biomass build up and rejuvenate dominant tussock grasses (Lunt & Morgan 1998) suggests that burning, mowing or grazing regimes that are designed so as to be appropriate for forb and other biodiversity values, can have a significant positive impact in reducing weediness in native grassland remnants. A significant component of weed control as a result of this action is likely to be due to the removal of dead leaf material above ground. This allows more rapid growth of green leaves, which in turn draws available nutrients from the soil up into the plant, and the reduced levels of available N and P will lead to a reduction in the size of annual grasses and flatweeds, and hence their impacts on biodiversity and visual amenity. The effectiveness of this mechanism for weed control in kangaroo grass dominated native grassland remnants is currently under investigation at Victoria University (C. Hocking *pers. comm.* 2000). It is widely observed that native grassland remnants that have frequent ecological burning or grazing have much lower weediness than long unburnt or ungrazed remnants (for example see the recommendations for management of native grasslands in Craigie & Hocking 1999, VNPA 1997 The extent to which this weediness is the result of the size of weeds, as opposed to their density or frequency, and how responsive weeds are to the levels of available soil N and P, need to be more widely tested.

Techniques for revegetating with native grassland species will need to find ways to minimise overwhelming weed growth, driven by the high soil nutrient levels typical in early stages of vegetation and to provide conditions and timing that will allow native species to preferentially take up the nutrients. Successful revegetation techniques will be those that lead in the long term to reductions in available soil N and P to such low levels that weeds are not able to gain the advantage above-ground.

An example of a revegetation technique which attempts to co-ordinate the release of nutrients with the preferential growth of native species over weeds is the ‘spray and hay’ technique for replacing serrated tussock (*Nassella trichotoma*) a noxious weed, with kangaroo grass in lowland grassland remnants (Phillips 2000, Phillips & Hocking 1996). In this method, serrated tussock is sprayed out in autumn using either Roundup (Monsanto glyphosate) or Nutrazine (Nufarm atrazine). Death of serrated tussock plants (like kangaroo grass, serrated tussock is a deep-rooted perennial grass) leads to significant rapid release of nutrients in the soil, as evidenced by the rampant growth in spring of a wide variety of flatweeds and annual grasses, compared to adjacent areas still dominated by serrated tussock. In the 'spray and hay' method, to protect the sprayed out site from major weed invasion, the site is covered with seed-bearing kangaroo grass hay. The awned seeds of kangaroo grass eventually work their way into the soil, and the hay is left in place during early autumn, to prevent weed growth. The hay is removed in late autumn (October-November) during the preferred time for kangaroo grass seed germination. The kangaroo grass seedlings grow rapidly, taking advantage of the high available soil nutrients and the lack of competition from annual weeds and flatweeds, which preferentially germinate in autumn. By the time of the autumn break, when flatweeds and annual grasses are ready to germinate, the kangaroo grass seedlings have come to dominate the site, having established a significant above-ground presence and having reduced the levels of available nutrients in the soil to the extent that any flatweeds and annual grasses that do establish in autumn do not grow to a sized which dominates the kangaroo grass seedlings.

It has recently been found (Phillips 2000, Hocking 2000) that the density of kangaroo grass seedlings is crucial to the success of the 'spray and hay' method in keeping weed growth in check during revegetation – if there are less than 30 seedlings m^{-2} then the weeds come to dominate the revegetation site in autumn and these shade out kangaroo grass seedlings to such an extent that the revegetation becomes unsuccessful (Hocking 2000). This 'density effect' is consistent with the importance of controlling available soil nutrient levels so that they advantage natives over exotics. The 'density effect' and its impacts on soil nutrient levels has also been observed in a study of the treatment of senescent grassland areas at Derrimut Grassland reserve. In 1998 Lunt and Morgan reported that kangaroo grass can senesce and die over a period of five to ten years if it is growing on the more productive soils in native grassland remnants. Measurements of soil nutrients under senescing kangaroo grass (S. Wijesuriya & C. Hocking 2000 – results not reported in this thesis) show elevated levels of available N and P compared with 'healthy' adjacent kangaroo grass areas.

These elevated nutrient levels are consistent with major growth of flatweeds and annual grasses in senescing kangaroo grass areas. Techniques currently under investigation to maximise the survival of senescing kangaroo grass plants and maintain densities of above 20 plants m^{-2} have been successful in keeping the size of weeds below a level which would swamp senescent kangaroo grass plants which have had dead leaf material removed by burning or mowing (C. Hocking *pers. comm.* 2000). Presumably the recovering kangaroo grass plants at densities of above 20 plants m^{-2} are able to keep available N and P at levels below which the flatweeds and annual grasses become dominant.

In conclusion, our models of lowland native grassland ecology need to incorporate the importance, and potential impacts on weediness, of available soil nutrient levels if techniques for management and repair are to be successful.

References

Adams D. E., Anderson R. C. and Collins S. L. (1982). Differential response of woody and herbaceous species to summer and winter burning in an Oklahoma grassland. Southwestern Naturalist. **27**, 55 - 61.

Adams M. and Attiwill P. (1986a). Nutrient cycling and nitrogen mineralization in Eucaliptus forests of South - eastern Australia. 1 Nutrient cycling and nitrogen turn over. Plant and Soil **92,** 319 - 339.

Adams M. and Attiwill P. (1986b). Nutrient cycling and nitrogen mineralization in Eucaliptus forests of South - eastern Australia. 2. Indices of nitrogen mineralization. Plant and Soil **92**, 341 - 362.

Adams M. A., Attivill P. M. and Polglase P. J. (1989). Availability of nitrogen and Phosphorus in forest soils in northeastern Tasmania. Biol. Fertil. Soils **8,** 212 - 218.

Adams M. A., Iser J., Keleher A. D. and Cheal D. C. (1994). Nitrogen and phosphorus availability and the role of fire in Heathlands at Wilsons Promontory. Aust. J. Bot. **42**, 269 - 281.

Aldous A. E. (1935). Management of Kansas permanent pastures. Kans. Agric. Exp. Sta. Bull. **272**, 44

Allen S. E. (1964). Chemical aspects of heather burning. J. Appl. Ecol. **1**, 347 - 367.

Allen S. E., Grimshaw H. M., Parkinson J. A. and Quarmby C. (1972). Chemical analysis of ecological materials. Blackwell scientific publications, Melbourne.

Arianoutsou M. and Margaris N. S. (1981). Fire induced nutrient losses in a phryganic (east Mediterranean) ecosystem. Microb. Ecol. **8**, 91 - 98.

Arianoutsou M. and Margaris N. S. (1982). Decomposers and the fire cycle in a phryganic (east Mediterranean) ecosystem. Microb. Ecol. **8**, 91 - 98.

Barker A. P. (1953). An ecological study of tussock grassland, Hunters Hill, South Canterbury. N. Z. Dept. Sci. Indus. Res. Bull. **107**, 58.

Belsky A. J. (1986). Revegetation of artificial disturbances in grasslands of the Serengeti National Park, Tanzania. Journal of Ecology **74**, 419 - 437.

Belski A. J. (1992). Effects of grazing, competition, disturbance and fire on species composition and diversity in grassland communities. Journal of Vegetation Science **3**, 187 - 200.

Bentley J. R. and Fenner R. L. (1958). Soil temperatures during burning related to post fire seedbeds on woodland range. J. For. **56**, 737 - 774.

Binkley D and Hart S. C (1989). The component of N availability assessments in forest soils. Adv. Soil Sci. **10**, 56 - 111.

Birch L. W. (1960). Nitrification in soils after different period of dryness. Plant and soil **12**, 81 - 96.

Birk E. M. and Vitousek P. M. (1986). Nitrogen availability and use efficiency in loblolly pine stands. Ecology **67**, 69 - 79.

Biswell H. H. (1956). Ecology of California grasslands. J. Range. Mgmt **9**, 19 -24.

Black C. A. (1968). Soil plant relation ship. 1 - 38. John Wiley & sons, Inc. USA.

Bonis A., Grubb P. J. and Coomes D. A. (1997). Requirements of gap demanding species in chalk grassland : reduction of root competition versus nutrient enrichment by animals. Journal of Ecology **85**, 625 - 633.

Bookman P. A. and Mack R. N. (1982). Root interaction between *Bromus tectorum* and *Poa pratensis*: a three dimensional analysis. Ecology **63**, 640 - 646.

Borman F. H. and Likens G. E. (1979). Pattern and process in a forested ecosystem. Springer - verlag, Berlin and New York.

Bowman D. M. J. S, Wilson B. A and Hooper R. J (1988). Response of *Eucaliptus* forests and woodland to four fire regimes at Munmarlary, Nothern Territory, Australia. J. Ecol. **76**, 215 - 232.

Brady C. N. (1984). The nature and properties of soils. 9th edition Macmillan publishing company, New York.

Bragg T. B. and Hulbert L. C. (1976). Woody plant invasion of unburned Kansas bluestem prairie. Journal of Range Management **29**, 19 - 24.

Bray R. H. and Kurtz L. T. (1945). Determination of total, organic and available forms of phosphorus in soils. Soil Sci. **59**, 39 - 45.

Bremner J. M. and Mulvaney C. S. (1982). Nitrogen total. In: Methods of soil analysis Part 2 (eds. C. A Black *et al.*). Agronomy No. 9 American society of Agronomy and Soil Science Society of America, Maidson, Wisconsin, USA.

Brynard A. M. (1965). The influence of veld burning on the vegetation and game of the Krugar National Park. In: Ecological studies in Southern Africa (ed. D. H. Davis) pp. 371 - 393. W. Junk, Netherlands.

Burke I. C. (1989). Control of N mineralization in a Sagebrush Steppe landscape. Ecology **70**, 1115 - 1126.

Burke M. J. W. and Grime J. P. (1996). An experimental study of plant community invasibility. Ecology **77**, 776 - 790.

Campbell M. H. (1960a). Identification of *Serrated tussock.* Agric. Gaz. N. S. W., Australia. **71**, 561 - 573.

Campbell M. H. (1960b). Only well managed sown pastures provide permanent tussock control. Agric. Gaz. N. S. W Australia. **71**, 9 - 19.

Chan C. W. (1980). Natural grasslands in Canberra. Their distribution phenology and effects of mowing. M.Sc. thesis. Aust. Nat. Univ. Canberra.

Charley J. L. and West N. E. (1977). Micro patterns of N mineralization activity in soils of some shrub-dominated semi-desert ecosystems of Utah. Soil Biol. Biochem. **9**: 357 - 365.

Christensen N. L. (1973). Fire and N cycle in Adenostoma Chaparral. Science **181**, 66 - 68.

Christensen N. L. and Peet R. K. (1984). The convergence during secondary forest succession. Journal of Ecology **72**, 25 - 36.

Clark F. E. and Woodmansee R. G. (1988). Nutrient cycling. Ecosystem of world. 137 - 146.

Connell J. H. (1978). Diversity in tropical rainforest and coral reefs. Science **199**, 1302 - 1310.

Cook L. (1939). A contribution to our information on grass burning. S. Afr. J. Sc. **36**, 270 - 282.

Cook L. (1965). Note upon burning experiments at Frankenwald, Transvall, South Africa. Tall Timbers Fire Ecol. Conf. **5**, 96 - 97.

Craigie V. and Stuwe J. (1992). Draft management plan. Derrimut grassland reserve. Department of Natural Resources and Environment.

Craigie V. and Hocking C. (1999). Down to grass roots: Proceedings of a conference on the management of grassy ecosystems, July 1998. Victoria University publication.

Daubenmire R. (1968). Ecology of fire in grasslands. adv. Ecol. Res. **5**, 209 - 266.

De Bano L. F. and Conrad C. E.(1978). The effect of fire on nutrients in a Chaparral ecosystem. Ecology **59**, 489 - 497.

Denslow J. S. (1980a). Gap partitioning among tropical rainforest trees. Biotropica **12**, Suppl. 47 - 55.

Denslow J. S. (1980b). Patterns of plant species diversity during succession under different disturbance regimes. Oecologia **46**, 18 - 21.

Dormaar J. F. (1992). Decomposition as a process in natural grasslands. In: Ecosystems of the world. (Ed. R. T. Coupl), **8A**, pp.121 - 133. ELSEVIER, Amsterdam.

Ehrenreich J. H. and Aikman J. M. (1957). Effect of burning on seedstalk production of native prairie grasses. Iowa Acad. Sci. Proc. **64**, 205 - 212.

Ehrenreich J. H. and Aikman J. M. (1963). An ecological study of certain management practices on native prairie in Iowa. Ecol. Monogr. **33**, 113 - 130.

Eno C. (1960). Nitrate production in the field by incubating the soil in polythene bags. Soil Sci. Soc. Am. Proc. **24**, 277 - 279.

Evans R. D. and Ehleringer J. R. (1993). A break in N cycle of aridlands; evidence from $\delta\ ^{15}N$ of soils. Oecologia **94**, 314 - 317.

Evans R. D. and Ehleringer J. R. (1994). Water and nitrogen dynamics in arid woodlands. Oecologia. **99**, 233 - 242.

Fisher H. J. (1974). Effect of nitrogen fertility on Kangaroo Grass pasture. Australian Journal of Experimental Agriculture and Animal Husbandry. **14**, 526 - 532.

Ford D. (1997). Interactions between vesicular - arbuscular mycorrhizae and nutrient addition on the growth of *Themeda triandra* and the promotion of exotic species in native Australian grasslands. B.Sc. (Hons.) thesis. 31 - 44. School of Botany, Latrobe University. Australia.

Garren K. H. (1943). Effects of fire on vegetation of the Southern United States. Bot. Rev. 9, 617 - 654.

Gibson D., Colquhoun I. and Greg S. P. (1985). A new method for measuring nutrient supply rates in soils using ion-exchange resins. In: Ecological interactions in soils; plants, microbes and animals (ed. A. H Fitter), pp. 73 - 79. Blakwell, Oxford.

Gibson D. J., Seasted T. R. and Briggs J. M.(1993). Management practices in tallgrass prairie. Large and small scale experimental effects on species composition. Journal of Applied Ecology **30**, 247 - 255.

Giesel J. T. (1976). Reproductive strategies as adaptations to life in temporally heterogeneous environments. Annu. Rev. Ecol. Syst. 7, 57 - 59.

Ginzo H. D., Collantes M. and Caso O. H. (1982). Fertilisation of native grassland in the depression del Rio Salado, province of Buenos Aires : herbage dry matter accumulation and botanical composition. Journal of Range Management **35**, 35 - 42.

Gough M. W. and Marrs R. H. (1990a). A comparison of soil fertility between seminatural and agricultural plant communities: implications for the creation of floristically rich grassland on abandoned agricultural land. Biol. Conserve. **51**, 83 - 96.

Gough M. W. and Marrs R. H. (1990b). Trend in soil chemistry and floristic associated with the establishment of a low - input meadow system on an arable clay soil in Essex England. Biol. Conserv. **52**, 135 - 146.

Grant S. A., Hunter R. F. and Cross C. (1963). The effects of muirburning *Molinia*-dominant communities. J. Br. Grassld Soc. **18**, 249 - 257.

Greene S. W. (1935a). Effect of annual grass fires on organic matter and other constituents of virgin long leaf pine soils. J. Agric. Res. 50, 809 - 822.

Green B. H. (1990). Agricultural intensification and the loss of habitat, species and amenity in British grasslands: a review of historical change and assessment of future prospects. Grass and Forage Science **45**, 365 - 372.

Grime T. P. (1979). Plant strategies and vegetational process. John Wiley, New York, USA.

Grime J. P., Hodgson J. G. and Hunt R. (1988). Comparative plant ecology: a functional approach to common British species. Unwin Hayman, London, England.

Groves R. H. (1965). Growth of Themeda tussock grasslands at St. A lbans, Victoria. Australian Journal of Botany. **13**, 291 - 302.

Groves R. H, Keraitisk K., Moore C. W. E (1973). Relative growth of *Themeda Australis* & *Poa labillardieri* in pots in response to phosphorus and nitrogen. Australian Journal of Botany. **23** : 951 - 963.

Groves R. H. and Williams O. B. (1981). Natural grasslands. In: Australian vegetation (ed. R. H.Groves), 293 - 315.Cambridge University press.

Gutterman Y. (1985). Flowering, seed development and the influences during seed maturation on seed germination of annual weeds. In: Weed physiology (ed. S. O. Duke), pp. 1 - 25. CRC press, Florida, USA.

Halstead R. L., Lapensee J. M. and Ivarson K. C. (1963). Mineralization of soil organic phosphorus with particular reference to the effect of lime. Canadian Jour. Soil Sci. **43**, 97 - 106.

Harmsen G. W. and Schreven D. A. (1955). Mineralization of organic matter in soil. Adv. Agron. 7, 299 - 398.

Haywood V. H. (1989). Patterns, extents and modes of invasions by terrestrial plants. In: Biological invasions (eds. Drake J. A, Mooney H. A, diCastri F, Groves R. H, Kruger F. J, Rejmanek M, Williamson M). John Wiley & sons, Chichester - New York.

Haywood V. H., Harborne J. B. and Turner B. L. (1977). The Biology and Chemistry of the Compositae. 1189. Academic Press, London.

Heady H. F. (1956). Changes in a California annual plant community invaded by manipulation of natural mulch. Ecology 37, 798 - 812.

Henderson M. and Hocking C. (1998). How do urban grasslands respond to fire and slashing? Effects on gaps and weediness. Management of grassy Ecosystems Conference proceedings. VUT. 38 - 43.

Hobbs R. J. (1987). Disturbance Regimes in Remnants of Natural Vegetation. In: Nature Conservation: The Role of Remnants of Native Vegetation (eds. D. A Sonders, G. W Arnold, A. A. Burbidgoand A. J. M. Hopkins), pp. 233 - 240. Surrey Beatty and Sons Pty. Limited.

Hocking C. (1996). Gungahlin grassland reserve. Mulegary ploughed area: report on proposal for field trials for replacement of oats with Kangaroo Grass and associated weed control. Report to ACT Parks and Conservation Service.

Hocking C. (2000). Modelling Life & Death in Grasslands - Roles for Nutrients & Light in Management, Repair & Recovery. Ecological Society of Australia, Annual Conference, Latrobe University, November.

Huenneke I. F., Hamburg S. P., Koide R., Mooney H. A. and Vitousek P. M. (1990). Effects of soil resources on plant invasion and community structure in Californian serpentine grassland. Ecology **71**, 478 - 491.

Hulbert L. C. (1973). Management of Konza prairie to approximate pre-white-man fire influences. In: Third Midwest prairie Conference proceedings (ed. L. C Hulbert). Kansas State University, Manhattan, Kansas.

Huston M. (1979). A general hypothesis of species diversity. Am. Nat. **113**, 81 - 101.

Jackson M. L. (1958). Soil chemical analysis. Prentice - Hall INC. Englewood Cliff N. J.

Joffre R. (1990). Plant and soil nitrogen dynamics in Mediterranean grasslands: a comparison of annual and perennial grasses. Oecologia **85**, 142 - 149.

Jeffrey L., Papendick R. I., Bezdick D. E. and Lynch J. M (1993). Organic matter dynamics and crop residue management. In: Soil microbial Ecology (ed. F. B. Meeting Jr), pp. 65 - 68. Marcel Dekkar Inc., New York.

Kaila A. (1965). Effect of liming on the mobilisation of soil phosphorus. Maat. Aikak. **37**, 243 - 254.

Keeny D. R. (1982). Nitrogen - Availability indices. In : Methods of soil analysis (eds. A. L Page, R. H Miller, D. R Keeny) 711 - 733. Part 2. American Society of Agronomy, Inc. Soil Science Society of America, Inc. Maidison, Wisconsin USA.

Keeny D. R. and Nelson D. W. (1982). Nitrogen - Inorganic forms. In : Methods of soil analysis (eds. A. L Page, R. H Miller, D. R Keeny), pp. 643 - 699. Part 2. American Society of Agronomy, Inc. Soil Science Society of America, Inc. Maidison, Wisconsin USA.

Kieft T. L, Soroke E. and Firestone M. K. (1987). Microbial biomass response to a rapid increase in water potential when dry soil is wetted. Soil Biol. Biochem. 19, 119 - 126.

Kirkpatrick J. E., McDougall K. and Hyde M. (1995). Australian most threatened ecosystems, the southern lowland native grasslands. Surrey Beatty & Sons. New South Wales, Australia.

Kirkpatrick J. E., Onus M. L. and Bromslow R. N. (1988). City parks and cemeteries. Tasmania's Remnant Grasslands and Grassy Woodlands. Tas. Con. Trust. Hobart.

Knapp A. K. and Seastedt T. R.(1986). Detritus accumulation limits productivity of tall grass prarie. Bioscience **36** (10), 662 - 668.

Knudsen D., Patterson G. A. and Pratt P. F. (1982). Lithium, Sodium and Potassium. In : Methods of soil analysis(eds. A. L Page, R. H Miller, D. R Keeny), pp. 643 - 699. American Society of Agronomy, Inc. Soil Science Society of America, Inc. Maidison, Wisconsin USA. Part 2.

Kochy M. and Wilson S. D. (1996). Litter decomposition and nitrogen dynamics in aspen forest and mixed grass prairie. Ecology **78(3)**, 732 - 739.

Kucera C. L. and Ehrenreich J. H. (1962). Some effects of annual burning on central Missouri Prairie. Ecology **43**, 334 - 336.

Lamp C. and Collet F. (1989). Field guide to weeds in Australia. 9 - 14. Inkata Press, Melbourne, 3rd edition.

Lamp C. A., Forbes S. J. and Cade J. W. (1990). Grasses of Temperate Australia. Inkata Press, Melbourne, Australia. 1 - 3.

Lamp D. (1980). Soil nitrogen mineralization in a secondary rainforest succession. Oecologia (Ber) 47, 257 - 263.

Lemon P. C. (1949). Successional response of herbs in the long leaf - slash pine type. Ecology **30**, 135 - 145.

Leps J., Osbornova - Kosinova J. and Rejmanek M. (1982). Community stability, complexity and species life history strategies. Vegetatio. **50**, 53 - 63.

Levin S. A. and Paire R. T. (1974). Disturbance, patch formation and community structure. Proc. Natl. Acad. Sci. USA **71**, 2744 - 2747.

Lodder M., Groves R. H. and Wittmark B. (1986). Native grasses - the missing link. Landscape Aust. **1**, 12 - 19.

Louis T. (1994). The effect of fire on nutrient losses and cycling in Quercus coccifera garrigue (Southern France). Oecologia 99, 379 - 386.

Lunt I. D. (1987). Effect of environment, land use and competition on vegetation patterns in a temperate (*Themeda triandra* Forsk.) grassland in Victoria, Australia. B.Sc (Hons.) thesis, Latrobe University, Melbourne.

Lunt I. D. (1990a). Species-area curves and growth-form spectra for some herb-rich woodlands in western Victoria, Australia. Aust. J. Ecol. **15**, 155 - 161.

Lunt I. D. (1990b). A floristic survey of the Derrimut grassland Reserve, Melbourne Victoria. Proc. R. Soc. Vic. **102**, 41 - 52.

Lunt I. D. (1990c). The soil seed bank of a long grazed *Themeda triandra* grassland in Victoria. Proc. R. Soc. Vic. **102**, 53 - 57.

Lunt I. D. (1990d). Impact of autumn fire on a long - grazed *Themeda triandra* (Kangaroo Grass) grassland: Implications for management of invaded, remnant vegetation's. Victorian naturalist. **107**, 45 - 51.

Lunt I. D. (1991). Management of remnant lowland grasslands and grassy woodlands for nature conservation: a review. Victorian Naturalists. **108: 3**, 56 - 66.

Lunt I. D. and Morgan J. (1998). Lessons from grassland management of Laverton North and Derrimut grassland Reserves: What can we learn for future grassland management. Management of Grassy Ecosystems Conference Proceedings. VUT, 69 - 74.

Maggs J. and Hewett B. (1993). Organic carbon and nutrients in surface soil from some primary rainforests, derived grasslands and secondary rainforests on the Atherton Tableland in North Eastern Queensland, Australia. J. Soil Res. **31**, 343 - 350.

Marrs R. H. (1993). Soil fertility and Nature conservation in Europe: Theoretical considerations and practical management solutions. Advances in Ecological studies **24**, 241 - 300.

Matson P. and Vitosek P. (1981). Nitrogen mineralization and nitrification potentials following clear cutting in the Hoosier National Forest, Indiana. For. Sci. **27**, 781 - 791.

Matthews S. (1999). The effects of burning and slashing management regimes in lowland native grasslands on the soil seed bank of the annual exotic grasses *Briza maxima* and *Vulpia bromoides*. Hons. thesis, School of Life Sciences and Technology, Victoria University, St. Albans, Victoria, Australia.

McDugall K. L. (1989). The Re-establishment of *Themeda triandra* (Kangaroo Grass): Implications for the restoration of grasslands. Department of Conservation, Forest and Lands, Victoria, Arthur Rylah Institute for Environmental Research, Technical Report services No. 89.

McDugall K. L. and Kirkpatrick J. B. (1994). Conservation of lowland native grasslands in south-eastern Australia. World Wide Fund for Nature Australia.

Mcgee C. E. (1976). Maximum soil temperatures on clear-cut forest land in western North Carolina. USDA For. Ser. Southeast. For. Exp. Stn., Res. Note SE - **237.**

Mcgraw J. B. and Chapin F. S. (1989). Competitive availability and adaptation to fertile and infertile soils in two Eriophorum species. Ecology. **70**, 736 - 739.

McLendon T. and Redente E. F. (1991). Nitrogen and Phosphorus effects on secondary succession dynamics on a semi-arid sagebrush site. Ecology **72**, 2016 - 2024.

McLendon T. and Redente E. F. (1992). Role of nitrogen availability in the transition from annual dominated seral communities. Symposium on Ecology, Management and Restoration of Intermountain Annual Rangelands, Boise, ID, May, 18 - 22.

Meiklejohn J. (1955). The effect of brush burning on the microflora of a Kenya upland soil. J. Soil Sci. **6**, 111 - 118.

Mes M. G. (1958). The influence of veld burning on the water, nitrogen and ash content of grasses. S. Afr. J. Sci. **54**, 83 - 86.

Michal P. W. (1970). Weeds of grasslands. Australian grasslands (ed. R. M. Moore), pp. 348 - 360. Australian National University press, Canberra.

Miller P. C. (1969a). Tests of solar radiation models in three forest canopies. Ecology **50**, 878 - 885.

Miller R. W. and Danahue R. L. (1995). Soil in our environment. 7th edition. Prentice Hall International (UK) Limited. London.

Milton W. (1947). The yield, botanical and chemical composition of natural hill herbage under manuring, controlled grazing and hay conditions. Journal of Ecology **63**, 621 - 626.

Mincler L. S. and Woerheide J. D. (1965). Reproduction of hardwood ten years after cutting as affected by site and opening size. J. For. **63**, 103 - 107.

Moore A. W. (1960). The influence of annual burning on a soil in the derived savanna zone of Nigeria. Internat. Congr. Soil Sci. Trans. 7th, 4, 257 - 264.

Moore C. W. E. (1964). Distribution of grasslands. In: Grasses and Grasslands (ed. C. Barnard), pp. 182 - 205. London, Macmillan.

Moore R. M. (1970). Australian grasslands. First edition, Australian national University, Canberra. Australia. 197 - 207.

Morcom L. (1990). The effects of grazing by introduce stock on the vegetation of Terrick State Park. Unpul. Rep. Dept. Cons. Forests Lands, Bendigo. Australia.

Morgan J. W. (1994). The ecology of Grasses and Grasslands in lowland Victoria. The Victorian Naturalists. **3**, 87 - 91.

Muir A. M. and Carr G. W. (1994). Evaluation of native grassland re-establishment report. Air liquide Australia pipeline establishment, Altona North, Victoria. Ecology Australia Pty. Ltd. Clifton Hill, Victoria, Australia.

Myrold D. D. (1987). Relationship between microbial biomass nitrogen and nitrogen availability index. Soil sc. Soc. Am. J. **51**, 1047 - 1049.

Myrold D. D. and Tiedje J. M. (1986). Simultaneous estimation of several nitrogen cycle rates using ^{15}N: Theory and application. Soil Biol. Biochem. **18**, 559 - 568.

Nicholas V.L.B.(1985). Tree falls, regrowth and community structure in tropical forests. In: The ecology of natural disturbance and patch dynamic (Eds. S. T. A. Picket & P. S. White), pp. 53 - 68. Academic press, INC. Orlando, Florida.

Nye P. H. and Greenland D. J. (1960). The soil under shifting cultivation. Tech. Rep. No. 51, Commonw. Bur. Soils, Farnham Royal, England.

O'Connor K. F. and Powell A. J. (1963). Studies in the management of Snow - tussock grassland. 1. The effects of burning, cutting and fertilizer on narrow - leaved Snow tussock (*Chionochloa rigida* (Raoul) Zotov) at a mid - altitude site in Canterbury, New Zealand. N. Z. Jl. agric. Res. **6**, 354 - 367.

Ojima D. S. (1987). The short term and long term effects of burning on tallgrass ecosystem properties and dynamics. Ph.D Dissertation. 98. Colo. State Univ. Ft. Collins Co. USA.

Ojima D. S., Schimel W. J. and Owensby C. E. (1994). Long and short term effects of fire on nitrogen cycling in tall grass prairie. Biogeochemistry **24**, 67 - 84.

Oliver C. D. (1981). Forest development in North America following major disturbances. For. Ecol. Manage. **3**, 153 - 168.

Olsen S. R. and Sommers L. E. (1982). Phosphorus. In: Methods of soil Analysis. Part 2. Chemical and Microbiological Properties. Second Edition. (Eds. A. L. Page, R. H. Miller and D. R. Keeny D. R). **No. 9 (part 2)**, pp. 403 - 427. AGRONOMY. American Society of Agronomy/Soil Science Society of America, Maidson, Wisconsin USA.

Owensby C. E. and Smith E. F. (1979). Fertilising and burning Flint Hills bluestem. Journal of Range Management **32**, 254 - 258.

Parrish J.A.D. and Bazzaz F.A (1985). Ontogenetic niche shifts in old- field annuals. Ecology **66**, 1296-1302.

Paul E. A., Smith J. L. and Norton J. M. (1986). The decomposition of ^{14}C and ^{15}N labelled cells in soil under anaerobic conditions. Agron. Abst. 1986 - 186.

Paul E. A. and Clark F. E. (1989). Soil microbiology and biochemistry. San diego: Academic press.

Petgel D. M (1987). Soil fertility and the composition of semi-natural grassland. In: Disturbance in grasslands (ed. J. Van Andel), pp. 51 - 61, Junk, Dordrecht.

Phillips A. and Hocking C (1996). A method for replacing serrated tussock (*Nassella trichotoma*) with weed-free kangaroo grass (*Themeda triandra*) in degraded native grassland remnants. Paper to 11th Australian Weds Conference, Melbourne (Aus). October.

Phillips A. (200). A method for replacing serrated tussock (*Nassella trichotoma*) with kangaroo grass (*Themeda triandra*) in lowland native grasslands. Master of Science thesis, submitted April.

Picket S. T. A. (1980). Non-equilibrium coexistence of plants. Bull. Torrey Bot. Club 107, 238 - 248.

Picket S. T. A. and White P. S. (1985). Patch dynamics a synthesis. In: The ecology of natural disturbance and patch dynamics. (eds. S. T. A Picket and P. S White), pp. 371 - 383. Academic Press Inc.. New York, USA.

Pigott C. D. and Taylor K. (1964). The distribution of some woodland herbs in relation to the supply of nitrogen and phosphorus in the soil. Journal of ecology **52** supplement, 175 - 185.

Platt W.J. (1975). The colonisation and formation of equilibrium plant species association on badge disturbances in a tall-grass prairie. Ecol. Monogr. **45**: 285-305.

Raison R. J. (1979). Modification of the soil environment by vegetation fires, with particular reference to nitrogen transformation: A review. Plant and Soil **50**, 73 - 108.

Raison R. J., Connell M.T. and Khanna P.K. (1987). Methodology for studying fluxes of soil mineral -N in-situ. Soil Biol.Biochem. **19** (5), 512-534.

Ramsay J. M. and Innes R.R. (1963). Some quantitative observations on the effects of fire on the Guinea savanna vegetation of nothern Ghana over a period of 11 years. Sols. Afr. **8**, 41 - 85.

Rayment G. E. and Higginson F. R. (1992). Australian Laboratory handbook of soil and water chemical methods. Inkata Press, Australia.

Reynolds G. H. and Bohning J. W. (1956). Effects of burning on desert grass - shrub range in Southern Arizona. Ecology **37**, 769 - 777.

Risser P. and Parton W. J. (1982). Ecological analysis of tallgrass prairie. Nitrogen cycle. Ecology **63**, 1342 - 1351.

Robertson D. (1985). Interrelationship between Kangaroo Grass, fire and vegetation dynamics at Gillibrand Hill Park, Victoria. Ph.D. thesis. University of Melbourne.

Ross D. J., Speir K. R. T. and Feltham C. W. (1997). Burning in a New Zealand snow- tussock grassland. Effect on soil microbial biomass and nitrogen and phosphorus availability. New Zealand Journal of Ecology, **21 (1)** 63 - 71.

Scarlett N. H and Parsons R. F. (1982). Rare plants of the Victorian plains. In: Species at Risk : Research in Australia 89 - 105 (eds. R. H Groves & W. D. L Ride), pp. 89 - 105. Aust. Acad. Sci., Canberra, Australia.

Schlesinger W. H. (1996). On the spatial pattern of soil nutrients in desert ecosystems. Ecology **77 (2),** 364 - 374.

Scott J. D. (1951). Conservation of vegetation in South Africa. Commonw. Bur. Pastures and Field Crops. Bull. **41**, 9 - 27.

Seastedt T. R., Briggs J. M. and Gibson D. J. (1991). Controls of nitrogen limitation in tallgrass prairie. Oecologia **87**, 72 - 99.

Sewell T. G. (1948). Burning of montane tussock grassland. N. Z. Jl. Agric. **77**, 263 - 269.

Sharp S. (1996). Management of native grasslands. Workshop report. Parks and Conservation, Canberra, Australia.

Skeen J. K. M (1956). Soil analysis as an aid to diagnosing deficiencies of phosphorus and potassium. Proc. Aust. Plant Nutrition Conf. Melb. **1,** 146 - 153.

Skeen J. N. (1976). Regeneration and survival of woody species in a naturally created forest opening. Bull. Torrey Bot. Club **103**, 259 - 265.

Smith C. J., Elston J. and Bunting A. H. (1971). The effects of cutting and fertilizer treatments on the yield and botanical composition of Chalk turf. J. Br. Grassland Soc. **26**, 213 - 219.

Smith R. T. and Taylor J. A. (1986). Bracken: Ecology, Land use and control Technology, Parthenon publishing, Carnforth.

Snaydon R. W. (1962). Micro distribution of *Trifolium repens* L. and its relation to soil factors. Journal of Ecology **50**, 133 - 143.

Solomon D. P., Berg L. R., Martin D. W. and Villee C. (1996). Biology. pp. 737 - 753. 4 th edition. Saunders College Publishing. New York.

Sprent J. I. (1987). The ecology of the nitrogen cycle. Cambridge University press, Cambridge, 2nd edition.

Specht R. L. (1963). Dark island heath (Ninety Mile Plain, South Australia). vii The effect of fertilisers on composition and growth, Australian Journal of Botany **11**: 62 - 66.

Sprugal D. G. (1985). Natural disturbance and ecosystem energetics. In: The ecology of natural disturbance and patch dynamics (eds. S. T. A Pickett & P. S White), pp. 335 - 352. Academic press Inc., New York.

Stanford G. and Epstein E.(1974). Nitrogen mineralization - water relations in soils. Soil Sci. Soc. Am. Proc. **38**, 103 - 107.

Strickland J. D. H. and Parson T. R. (1977). Determination of ammonium. In: practical hand book of seawater analysis. Chemical Oceanography (J. P.Riley & G. Skirrow), pp 81 - 89. Academic press, London. Fisheries Research Board of Canada. Bulletin. volumes 1 & 2. 2nd edition.

Stuwe J. and Parsons R. F. (1977). *Themeda australis* grasslands on the Basalt plains, Victoria: floristic and management effects. Aust. J. Ecol. **2**, 467 - 476.

Suckling G. and MacFarlane M. A (1984). The effects of fire on fauna. In: a review. Fighting fire with fire (ed. E. M. Ealey), pp. 28 - 35. Aristoc Press, Melbourne.

Stuwe J. and Darsons R.F. (1977). Themeda australis grasslands on the Basalt Plains, Victoria: floristic and management effects. Australian Journal of Ecology. **2**: 467- 476.

Swift L. W., Swank W. T., Mankin J. B., Luxmoore R. J. and Golstein R. A. (1975). Simulation of evapotranspiration and drainage from mature and clearcut deciduous forests and young pine plantations. Water Resour. Res. **11**, 667 - 673.

Theron J. J. (1951). The influence of plants on the mineralization of nitrogen and maintenance of organic matter in the soil. J. Agric. Sci. **41**, 289 - 296.

Theron J. J. and Haylett D. G. (1953). The regeneration of soil humas under a grass ley. Emp. J. Exp. Agric. **21**, 86 - 98.

Thompson E. J., Olivera A. L. F., Moster U. S. and Black C. A. (1960). Evaluation of laboratory indexes of absorption of soil phosphorus by plants. II, Plant Soil **13**, 28 - 38.

Tilman G.D. (1984). Plant dominance along an experimental nutrient gradient. Ecological Society of America. **65 (5)**, 1445-1453.

Tilman G. D. (1986). Nitrogen Limited growth in plants from different successional stages. Ecology **67(2)**, 555-563.

Titlyanova A. A., Zlotin R. I. and French N. R. (1990). Changes in structure and function of temperate zone grasslands under the influence of man. In: Ecosystems of the world, Managed grasslands, regional studies (ed. A. I. Breymeyer), pp. 301 - 334. 17 A, ELSEVIER science, The Netherlands.

Trabaud L. (1983 b). The effects of different fire regimes on soil nutrient levels in *Quercus coccifera* garrigue. In: Mediterranean type ecosystems. Role of nutrients (eds. F. J Kruger, D. T Mitchell and J. U. M Jarvis), pp. 233 - 243. Springer, Berlin, Heidelberg, New York.

Trabaud L. (1994). The effect of fire on nutrient losses and cycling in *Quercus coccifera* garrigue (Southern France). Oecologia **99**, 379 - 386.

Vitousek P. M. (1985). Community turnover and Ecosystem nutrient dynamics. In: The ecology of natural disturbance and patch dynamics (eds. S. T. A Pickett & P. S White). pp. 325 - 333. Academic press Inc. New York.

Vitousek P.M., Gosz J.R., Grier C.C., Melillo J.M. and Reiners W.A. (1982). A comparative analysis of potential nitrification and nitrate mobility in forest ecosystems. Ecological Monographs **52**, 155-177.

VNPA- Victorian National Parks Association, 1997. Looking after native grasslands. and grassy woodlands. VNPA Inc. publication.

Waksman S. A. (1927). Principles of soil microbiology. 897. Williams and Wilkins Co. Baltimore.

Wang C., Attiwil P.M. and Adams M.A. (1996). Fertiliser impacts on the understorey of a regenerating mountain ash (*Eucalyptus regnans* F. Muell.) forest, Victoria. Australian Journal of Ecology **21**, 459- 463.

Weaver J. E. and Rowland N. W. (1952). Effects of excessive natural mulch on development, yield and structure of native grassland. Bot. Gaz. **114**, 1 - 19.

Weins J. A. (1976). Population responses to patchy environments. Annu. Rev. Ecol. Syst. **7**, 81 - 120.

Wells T. C. E. (1980). Management options for lowland grassland. In: Amenity Grassland: An Ecological Perspective (ed. I. H Rorison & R Hunt), pp. 175 - 196, Wiley, Chichester.

Wendin D. A. and Tilman D. (1992). Nitrogen cycling, plant competition and the stability of tall grass prairie. In: Proceedings of the 12th North American prairie conference (eds. D. D Smith & Jacobs C. A), pp. 5 - 8. University of nothern Iowa press. Cedar Falls, Iowa.

West O. ((1965). Fire in vegetation and its use in pasture management, with special reference to tropical and subtropical Africa. Commonw. Bur. Pastures and Crops, Bucks, Engl. pp. 53.

White P. S. (1979). Pattern, process and natural disturbance in vegetation. Bot. Rev. **45**, 229 - 299.

Whitmore T. C. (1975). Tropical Rainforest of the Far East. Oxford Univ. Press (Clarendon), London and New York.

Whitmore T. C. (1978). Gaps in the forest canopy. In: Tropical trees as living system (eds. P. B Tomlinson and M. H Zimmermann). pp. 639 - 655. Cambridge University Press. London and New York.

Whitmore T. C. (1982). On pattern and process in forest. In: The plant Community as a working mechanism. (ed. J. Newman). pp. 45 - 57. Blackwell, Oxford.

Williams C. H. and Andrew C. H. (1970). Mineral nutrition of pastures. In: *Australian grasslands* (ed. R. Milton Moore), pp. 322 - 338. Australian National University press, Australia.

Williams D., Osborne W., Georges A. and Holloway S. (1995). Principles and strategic options for the conservation of native grasslands and their threatened fauna in Gungahlin, A.C.T. Applied Ecology Research group, University of Canberra.

Willis A. J. and Yemm E. W. (1961). Braunton Burrows mineral nutrient status of the dune soils. J. Ecol. 49, 377 - 390.

Willis J.H. (1964). Vegetation of the Basalt Plains in western Victoria. Prceedings of the Royal Society of Victoria. **77**, 397 - 418.

Wills A. and Yemn E. (1967). Braunton Burrows: mineral nutrient status of the dune soils. Journal of Ecology **49**, 377-390.

Whittaker R.H. (1965). Dominance & diversity in land plant communities. Science **147**, 250-60.

Whittaker R. H. and Niering W. A. (1975). Vegetation of the Santa Catalina mountains, Arizona. V. Biomass, production and diversity along the elevation gradient. Ecology **56**, 771 - 790.

Woodmansee R. G. and Wallach L. S. (1981). Effect of fire on biogeochemical cycles. Ecol. Bull. **33**, 649 - 669.

Zar J. H. (1996). Biostatistical analysis. 3rd edtion, Prentice Hall, Inc. Simon & Schuster/ A Viacom Company Upper Saddle River, New Jersey 07458.

Appendix 1

List of instruments used

1. Atomic Absorption Spectrometer

SpectrAA. 300/400

Varian, 1988.

2. UV-Visible Spectrophotometer

CARY - 1

Varian, 1990.

3. Flow Injection Auto Analyser

AQUATEC - 5400

Tecator.

Appendix 2

Abstracts of papers and posters presented

1. Ecological society of Australia (ESA) Conference - University of Tasmania, Hobart.

Sardha Wijesuriya and Colin Hocking, Dept. of Environmental Management, Victoria University of Technology, PO BOX 14428, Melbourne, VIC. **Effect of soil disturbance, gap size and type of treatment on weed colonization in a *Themeda triandra* dominated western (basalt) plains grassland remnant.**

Little research has been undertaken in Australia into the status and dynamics of soil nutrients and other soil parameters in native grassland remnants. Recent overseas studies have shown that changes in nutrients after soil disturbance in grasslands and shrublands may be important in predicting the subsequent pattern of colonization of the disturbed area by grasses and forbs. Similar mechanisms may contribute to the rapid colonization of native grassland soil by weeds after disturbance. Preliminary studies have been undertaken at St Albans on the western edge of Melbourne, of the patterns of weed colonization in small gaps created by disturbing the soil in various ways in *Themeda triandra* (Forsk.) dominated grassland . Nitrogen, in the form of ammonium nitrate, has been added to some gaps to increase total nitrogen. Sucrose was added to other gaps to stimulate microbial activity, thereby decreasing nitrogen levels in the soil.

Results of these trials are consistent with the suggestion that the levels of nutrients in the soil after disturbance are related to the type and extent of colonization by weeds. Gaps to which nitrogen had been added exhibited significantly higher levels of annual weeds than untreated gaps. Smaller gaps contained fewer weeds per unit area than larger gaps. In plots where roots had been removed from soil during disturbance, overall levels of weed colonization were low. This suggests that breakdown of root material in disturbed soil may abe contributing to the potential for weed invasion. Levels of exchangeable nitrogen and phosphorus in soil after disturbance, in combination with additions of ammonium nitrate or sugar, will be presented.

2. Ecological Society of Australia (ESA) Conference - Charles Stuart University, Albury, New South Wales.

Sardha Wijesuriya and Colin Hocking Dept. of Biological and Food Sciences, Victoria University of Technology. **The effects of biomass reduction (burning and mowing) on grassland soil nutrients - preliminary results.** ☯ 🖂

Disturbances such as digging, fire, and fertilising affect weed invasion in lowland native grassland remnants, but reasons for this are unknown. A project is underway to examine the links between weed invasion and available soil nutrient levels in two grassland reserves on the Victorian western basalt plains. A series of burning and mowing treatments were carried out in autumn and spring 1996. Results analysed to date for plots burnt or mown in autumn show that: 1. The balance between Ammonium-N and Nitrate-N changes during the year, with Ammonium-N becoming more prominent over Nitrate-N as soil became waterlogged. 2. Untreated and mown plots showed the same patterns of seasonal change in Nitrate-N and Ammonium-N. Burnt plots on the other hand showed different seasonal patterns of change for Nitrate-N and Ammonium-N. In addition the seasonal patterns of available nitrogen were different for each of the two sites. 3. Seasonal patterns of available phosphorus were similar across treatments, but different at each site. 4. The seasonal patterns of available potassium were not significantly different between treatments. These results will be discussed in the context of floristic data showing differences in weed content between treatments.

[redacted]rassy Ecosystems - Victoria University of Technology, St.

Thursday 9 July 2.00 - 2.20

Why do weeds grow when you dig up native grasslands? The effects of physical soil disturbance on available nutrient levels, mineralization and weed invasion in native grasslands.

Sardha Wijesuriya and Colin Hocking
School of Life Sciences and Technology, Victoria University of Technology, St. Albans.

Our study determined the levels of available soil nutrients in artificially disturbed small plots at Derrimu grassland reserve and how available soil nutrient levels affect the growth of weeds. Soil was remove from small round plots (1m diameter) in two steps: 1cm - 7cm and 7cm - 15cm. Each layer was kept separate. Each was homogenized and returned to the hole in appropriate order. Soil was initially analysed for available nutrients (NO_3^- - N; NH_4^+ - N; $H_2PO_4^{2-}$ - P and K^+) 14 days after digging and at days and 70 days thereafter. After 70 days total available N was about 10 times greater in dug plots than in control plots and mineralizable N (in-situ) in dug plots was about 5 times greater than in control (ie. undug) plots. The levels of available P in dug plots was about 1.5 times higher than in control plot Interestingly, the rate of mineralization of P was not significantly different between treatments.

The analysis of above ground biomass of plants growing on disturbed plots showed higher biomass of thistles and annual grasses in dug plots than undug (ie. control) plots. These were the dominant plant types on all disturbed plots. The addition of nutrients (N&P) to dug plots, increased above ground vegetative biomass. Conversely the addition of organic C (as sugar) decreased above ground vegetative biomass, compared to controls. Differences in overall biomass of weeds on the plots was paralleled by differences in biomass of annual grasses and thistles. We suggest that on disturbed soil in native grasslands the release of nutrients following plant death contributes substantially to the the massive increase in weediness following disturbance.

4. New Zealand Ecological Society and Ecological Society of Australia - University of Otago, New Zealand.

☺ ✉ Sardha Wijesuriya and Colin Hocking School of Life Sciences and Technology, Victoria University of Technology, St. Albans campus, P. O. Box 14428, MMC 14428, Australia. **Soil nutrient status and weed invasion in physically disturbed native grasslands.**

This study investigated the levels of available soil nutrients in artificially disturbed small plots and how available soil nutrient levels affect the growth of weeds. Soil was removed from small round plots (1m diameter) in two steps: 1cm- 7cm and 7cm- 15cm. Each layer was kept separate. Each was homogenised and returned to the hole in appropriate order. Soil was initially analysed for available nutrients(NO_3^- - N; NH_4^+- N; $H_2PO_4^-$ - P and K^+) 14 days after digging and at 34 days and 70 days thereafter. After 70 days total available N was 10 times greater in dug plots than in control plots and mineralizable N (in-situ) in dug plots was about 5 times greater than in control (undug) plots. The levels of available P in dug plots was 1.5 times higher than in control plots. Interestingly, the rate of mineralization of P was not significantly different between treatments. The available K was significantly higher in dug plots than in controls. The analysis of above ground biomass of plants growing on disturbed plots showed higher biomass of thistles and annual grasses in dug plots than undug (ie. control) plots. These were the dominant plant types on all disturbed plots. The addition of nutrients (N and P) to dug plots, increased above ground vegetative biomass. Conversely the addition of organic C (as sugar) decreased above ground vegetative biomass, compared to controls. Differences in overall biomass of weeds on the plots was paralleled by differences in biomass of annual grasses and thistles. We suggest that on disturbed soils in native grasslands the release of nutrients following plant death contributes substantially to the massive increase in weediness following disturbance.

Appendix 3. Details of statistical analysis

ANOVA - Multiple comparisons (Tukey honestly significant difference test)

Statistical analysis refers to data shown in Fig. 5.2.

plant type	dug	dug + N	dug + P	dug + N + P	dug + C
Themeda	a 0.23 (0.13)	a 0.37 (0.14)	a 0.29 (0.07)	b 0.75 (0.25)	a 0.27 (0.14)
annual grasses	a 1.39 (0.33)	a 0.8 (0.33)	b 2.5 (0.8)	b 2.18 (0.8)	a 1.0 (0.44)
Thistles & other broad leaved weeds	a 4.26 (1.12)	a 2.56 (0.87)	a 3.49 (1.01)	b 11.11 (4.5)	c 1.26 (0.4)
Others	a 0.15 (0.09)	a 0.34 (0.31)	a 0.32 (0.19)	a 0.65 (0.41)	a 0.3 (0.24)
Total	a 6.03 (1.67)	ac 4.06 (1.65)	a 6.61 (2.1)	b 14.69 (5.6)	c 2.83 (1.1)

Mean values of plant biomass. Standard errors are in brackets ($n = 8$).

Mean values with different letters differ significantly($p < 0.05$ at least) between different nutrient treatments for each plant type. Means sharing same letters are not significantly different ($p > 0.01$ at least). The power of significance is given with appropriate results.

Statistical analysis refers to data shown in Fig. 6.1.

plant type	dug (large)	dug (small)
Themeda	a 11.6 (4.4)	a 6.54 (2.3)
Annual grasses	a 149.2 (19.32)	b 101.2 (14.21)
Thistles & other broad leaved weeds	a 2 (0.66)	a 0.8 (0.56)
Others	a 8.85 (2.79)	a 8.83 (3.69)
Total	a 171.65 (27.17)	b 117.37 (20.76)

Mean values of plant biomass. Standard errors are in brackets ($n = 8$).
Mean values with different letters differ significantly ($p < 0.05$ at least) between dug large and dug small treatments for each plant type. Means sharing same letters are not significantly different ($p > 0.01$ at least). The power of significance is given with appropriate results.

Statistical analysis refers to data shown in Fig. 7.3

plant type	burnt (control)	N	P	N + P	sugar (C)
Themeda	a 26.67 (3.90)	a 25.27 (3.86)	a 21.28 (2.66)	a 18.62 (5.32)	a 23.94 (2.67)
annual grasses	a 17.29 (3.93)	a 20.80 (6.65)	a 25.27 (7.98)	b 67.83(14.63)	c 10.64 (2.69)
Thistles & other broad leaved weeds	a 2.67 (1.3)	a 2.42 (1.00)	a 2.52 (1.5)	a 9.31 (6.65)	b 0.70 (0.25)
Others	a 15.96 (5.32)	a 17.29 (5.3)	a 10.64 (3.98)	a 19.95 (5.31)	a 10.64 (3.33)
Total	a 63.84 (13.30)	a 66.50 (15.96)	ac 61.18 (14.63)	b 117.04 (30.59)	c 41.23 (7.98)

Mean values of plant biomass. Standard errors are in brackets ($n = 8$).

Mean values with different letters differ significantly($p < 0.05$ at least) between different nutrient treatments for each plant type. Means sharing same letters are not significantly different ($p > 0.01$ at least). The power of significance is given with appropriate results.

Statistical analysis refers to data shown in Fig. 7.4.

plant type	burnt (control)	N	P	N + P	sugar (C)
Themeda	a 25.00 (4.13)	a 23.85 (3.21)	a 20.51 (3.33)	a 18.67 (3.42)	a 20.52 (2.82)
B. maxima	a 8.34 (2.75)	a 12.43 (4.35)	a 11.05 (3.67)	b 26.08 (5.15)	c 5.38 (1.26)
B. minor	a 1.67 (0.67)	a 1.31 (0.52)	a 1.25 (0.71)	b 4.38 (2.33)	a 0.56 (0.02)
V. bromoides	a 4.72 (1.31)	a 4.32 (1.38)	b 10.65 (2.71)	c 28.77 (9.82)	d 2.46 (0.55)

Mean values of plant biomass. Standard errors are in brackets (n = 8).

Mean values with different letters differ significantly($p < 0.05$ at least) between different nutrient treatments for each plant type. Means sharing same letters are not significantly different ($p > 0.01$ at least). The power of significance is given with appropriate results.

Statistical analysis refers to data shown in Fig. 7.5

plant type	burnt (control)	N	P	N + P	sugar (C)
Themeda	ab 34.26 (4.38)	a 30.26 (4.84)	a 28.24 (2.98)	a 24.76 (1.92)	b 40.00 (3.38)
annual grasses	a 35.26 (8.98)	a 35.52 (10.94)	a 46.26 (11.02)	a 44.78 (10.24)	a 28.24 (6.34)
Thistles & other broad leaved weeds	a 9.02 (4.4)	a 13.02 (7.16)	a 7.72 (2.66)	a 7.24 (5.26)	a 7.78 (1.78)
Others	a 1.48 (1.24)	a 3.00 (2.08)	a 1.22 (1.12)	a 1.74 (1.36)	a 2.48 (1.78)
Total	a 80.02 (18.98)	a 78.8 (25.02)	a 83.44 (18.68)	a 76.02 (20.18)	a 78.30 (13.5)

Mean values of number of plants (m^{-2}). Standard errors are in brackets ($n = 8$).

Mean values with different letters differ significantly($p < 0.05$ at least) between different nutrient treatments for each plant type. Means sharing same letters are not significantly different ($p > 0.01$ at least). The power of significance is given with appropriate results.

Statistical analysis refers to data shown in Fig. 7.6.

plant type	burnt (control)	N	P	N + P	C (sugar)
Themeda	a 0.73 (0.11)	a 0.79 (0.12)	ab 0.70 (0.1)	ab 0.71 (0.17)	b 0.52 (0.09)
Annual grasses	a 0.44 (0.08)	a 0.54 (0.12)	a 0.52 (0.10)	b 1.40 (0.28)	c 0.20 (0.05)
Thistles and other broad leaved weeds	a 0.25 (0.06)	a 0.13 (0.08)	a 0.17 (0.07)	b 1.21 (0.34)	a 0.09 (0.03)

Mean values of biomass / number of plants. Standard errors are in brackets ($n = 8$). Mean values with different letters differ significantly($p < 0.05$ at least) between different nutrient treatments for each plant type. Means sharing same letters are not significantly different ($p > 0.01$ at least). The power of significance is given with appropriate results.