A STUDY ON THE MAIN FACTORS AFFECTING THE COMPETITIVE GROWTH OF *THEMEDA TRIANDRA* (KANGAROO GRASS) AND THE INTRODUCED WEED, *NASSELLA TRICHOTOMA* (SERRATED TUSSOCK).

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CONTENTS

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ABSTRACT:

Little published information is available on the levels of soil nutrients and soil moisture on remnants of native (Basalt Plains) grasslands.

This study investigated the association of soil nutrients and moisture with stands of the native grass Themeda *triandra* (Kangaroo Grass) and the noxious weed *Nassella trichotoma* (Serrated Tussock). Both mature and immature (i.e. recently burnt) stands of *T. triandra* and *N. trichotoma* were investigated, on disturbed and undisturbed soil.

No significant differences for the nutrients (Total Kjeldahl Nitrogen and Total Phosphorus) between soil under *T. triandra* and *N. trichotoma* on either the disturbed or undisturbed sites was found.

Soil moisture was found to be significantly different between mature stands of *T. triandra* and *N. trichotoma*, with the *N. trichotoma* stand having significantly lower soil moisture. The results suggest that one of the factors that allows *N.trichotoma* to invade native remnant grasslands and disturbed sites, and to resist the re-establishment of T. triandra on invaded sites might be the need of *T. triandra* for moist soil for seed germination.

The general aim of this study was to undertake an initial investigation of the environmental factors that might advantage *Nassella trichotoma* in invading remnant grassland sites at the expense of *Themeda triandra*.

1. BACKGROUND TO THE STUDY

1.1 HISTORY OF NATIVE GRASSLANDS IN VICTORIA

Prior to pastoral settlement of the Victorian native grasslands beginning in the 1830's, native grasslands and grassy woodlands* covered 34% of Victoria (Cons. & Env. 1992; Cons. & Nat. Res. 1993a & 1993b) (see figure 1a). A major component of these grasslands was the Victorian (Basalt Plains) grassland which covered 10% of Victoria (see figure 1b). This ecosystem was dominated by *Themeda triandra* or Kangaroo Grass (formerly known as *Themeda australis* - see figure 2) (Stuwe & Parsons 1977; Groves 1965).

The agricultural practices of the settlers lead to the degradation and loss of many areas of native grasslands through their conversion to exotic pastures, crops or urban development (Groves 1965; Lunt 1991).

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* Grasslands and grassy woodlands have been shown to have a close association. With the understory species of grassy woodlands comprising of the same floral composition as adjacent grasslands. In this report the term grasslands is used to refer to both open grasslands and the understory of grassy woodlands (Frood & Calder 1987).

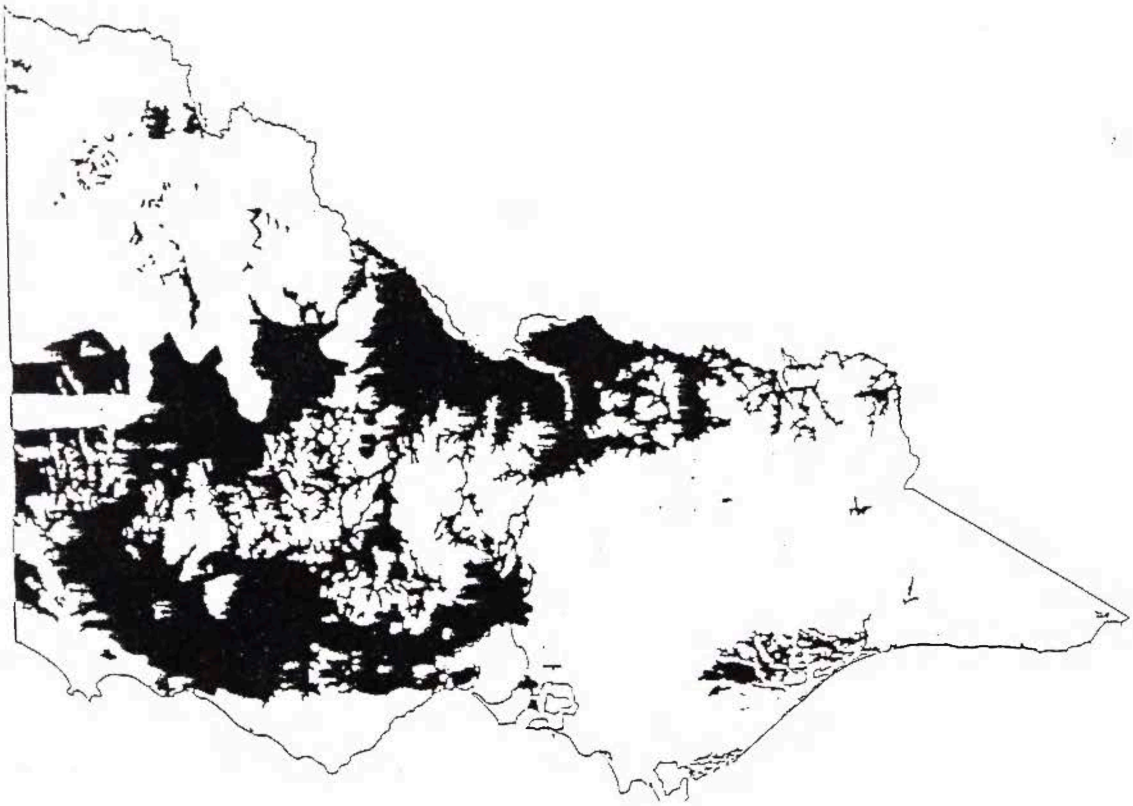

Figure 1a: Pre - settlement distribution of native grasslands and grassy woodlands in Victoria (Anon 1992).

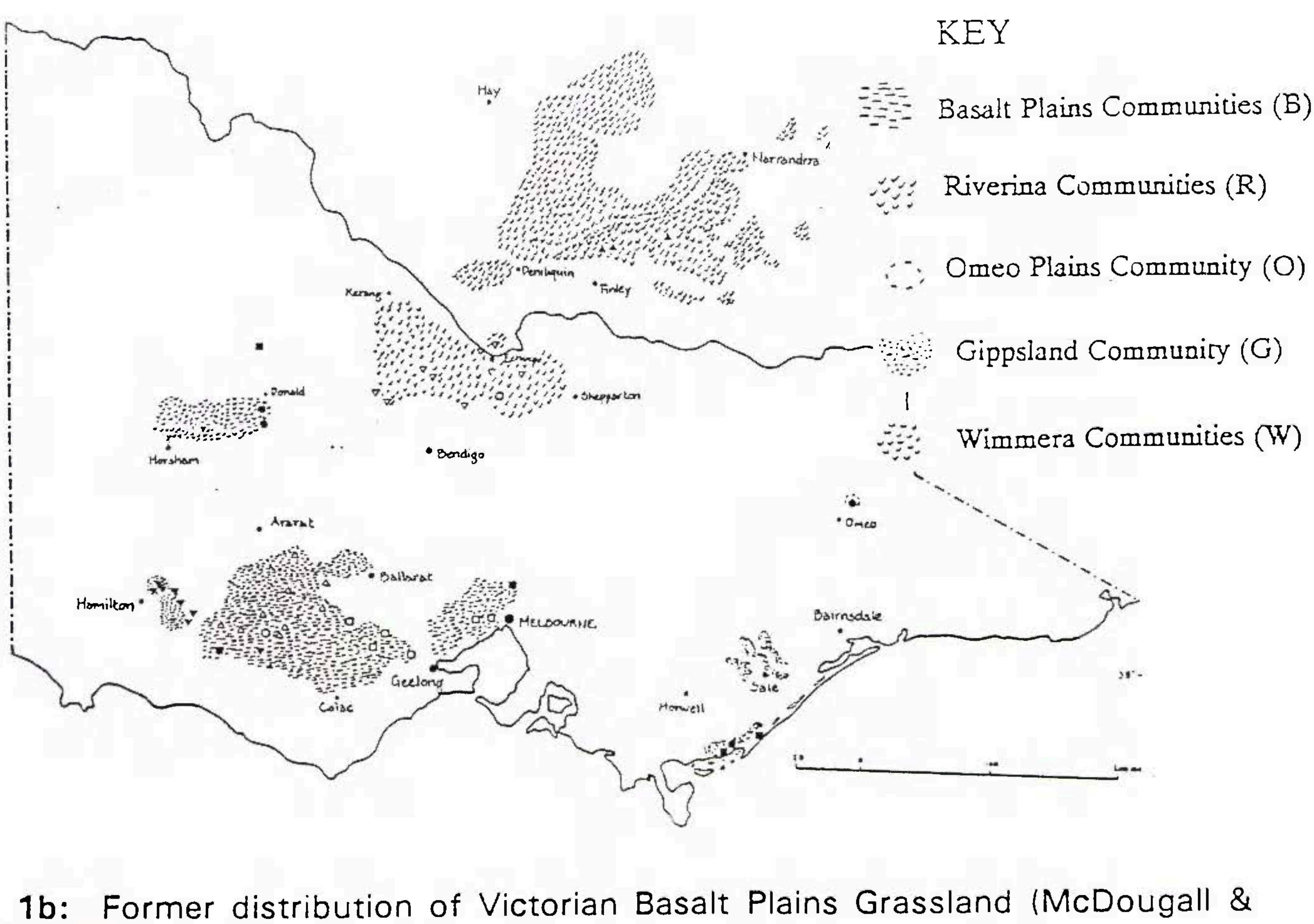

Figure 1b: Former distribution of Victorian Basalt Plains Grassland (McDougall & Kirkpatric 1994).

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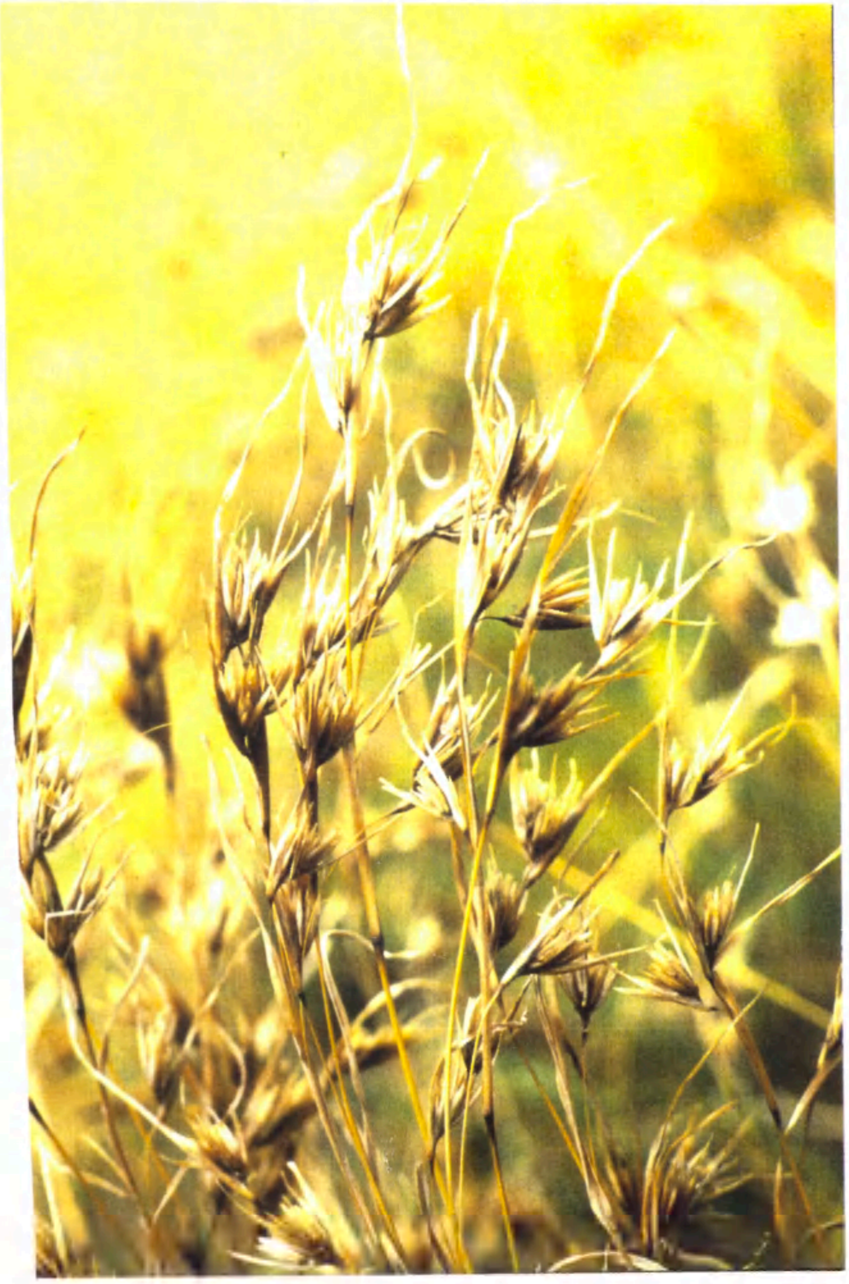


Figure 2: *Themeda triandra* - the dominant native grass of the Western Basalt Plains Grassland.

Figure 3: *Nassella trichotoma* - introduced weed which is causing a major problem in native grasslands.

Over much of this period of settlement (at least the last 100 years) agriculturalists have predicted the degradation and loss of the Victorian native grasslands due to the lack of care taken in the way that they have been used and managed (Turner 1891; Sutton 1916). Collequial

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 ecotype in Australia with less than 0.5% remaining (Lunt 1991; Anon 1993a & 1993b). 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). where are these refs ???

With the loss of native grasslands has come the national, regional and local extinction of many species of invertebrates, mammals and plants (Craigie & Stuwe 1992; Anon 1993a & 1993b). Again Genetic diversity has always been a vital component of any natural communities ability to weather environmental changes's. The drastic reduction and degradation of native grasslands has reduced the biodiversity of the grasslands and the gene pool available. This has resulted in the entire Western Basalt Plains Grassland community being listed in the *Flora and Fauna Guarantee Act* (1988) as a threatened community. Ref

1.2 *THEMEDA* DOMINATED GRASSLANDS

Themeda triandra is a member of the grass family *Poaceae*. It is a dense tussocky warm-seasonal perennial which normally flowers between November and January (Groves 1975; Scarlett 1992) and has a C4 photosynthesis pathway (Stuwe & Parsons 1977; Groves 1975; Culvenor 1981).

It is found in all the mainland states of Australia as well as Tasmania and Papua New Guinea.

what is found

Themeda-dominated grasslands also contain a characteristically ~~contain a~~ wide range of other grasses and forb species, growing ~~amongst the *Themeda*~~. Many of these minor grasses and forbs are greatly reduced in number with even minimal disturbance of grasslands, such as grazing or fertiliser application refs. In their place grow introduced species which are better adapted to hard hoofed animals. It has been suggested that native grass replacement by weeds is due to 3 factors: selective grazing, physical disturbance associated with hoofed animals (Muir 1992), and higher nutrient concentration of improved pastures (Groves 1975).

1.3 FIRE AS A COMPONENT OF GRASSLAND ECOSYSTEMS

is this supposed to be fire??

Prior to European settlement and the associated grazing of soft footed animals (eg. kangaroos), was used for millenia to manage native grasslands and maintain species diversity. Fire was initially used by Aboriginal groups to clear unwanted vegetation, promote regrowth and trigger the release of seeds for food refs. Forty thousand years of Aboriginal burning regimes led to many native grassland plants becoming highly adapted to frequent fires. trite. You must use refs to support

After European settlement there were fewer fires due to acute fire suppression and because the fuel was grazed by domestic, indigenous and feral herbivores (Groves 1981). In recent times controlled fires have been increasingly used to limit the risk of an uncontrolled fire by reducing the accumulation of fuel. This needs more explanation. ie what has/is the result, real or possible of altered fire regime??

In native grasslands fire acts to promote many of the smaller herb species that would otherwise be out competed by the *Themeda* (Craigie & Stuwe 1992). colloquial Burning has generally been considered a good management tool for grasslands that are relatively free of exotic species (Kirkpatric 1986; Lunt 1990; Anon 1992), — where is this?? as it is not as discriminating as grazing and it supplies bare ground for the growth of seedlings. However there is some uncertainty about the use of fire as a management tool. Lunt (1990) observed that after fires in some grassland areas that had been degenerated by grazing and the invasion of exotic species, more of the exotic species often regenerated or became established than the native species.

There is an urgent need for more research into methods of flora management in native grasslands which will promote growth of native species ahead of the exotic species.

1.4 COMPETITION FROM WEEDS

The invasion of native grasslands by introduced weeds is a widespread problem throughout the world. Weeds influence native grasslands by competing with native species for available space, nutrients and moisture (Cons. & Env. 1992).

In the Victorian Basalt Plains Grassland the native vegetation is resistant to invasion when left undisturbed. However native grassland vegetation is easily replaced by exotic plants when it is exposed to soil disturbances, intensive grazing or increased nutrients (Craigie & Stuwe 1992). Often a combination of these factors has disastrous effects on remnant grassland stands.

Nassella trichotoma (Serrated Tussock - see figure 3) ranks amongst the most problematic weeds in these grasslands (Craigie and Stuwe 1992), as well as on pasture land (see below). It originated in South America and was spread via the import of wool and hay from there to South Africa, New Zealand and Australia.

Since its arrival in Australia (around 1900) *N. trichotoma* has infested much of the southeast area of New South Wales as well as smaller areas to the north and parts of Tasmania, King Island and Victoria (Campbell 1982) (see figures 4 & 5), and has been declared a noxious weed.

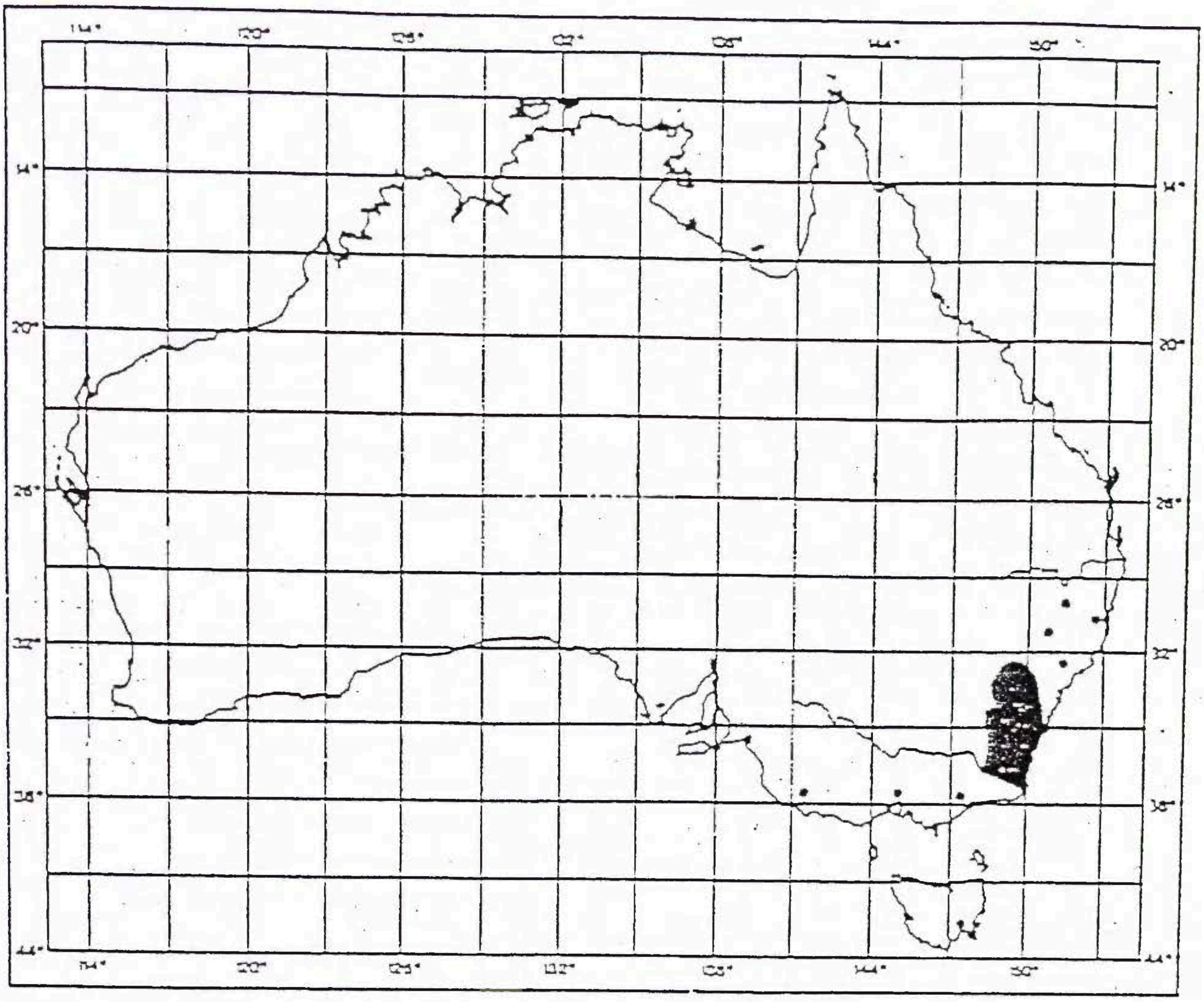


Figure 4: The distribution of *Nassella trichotoma* in Australia (Campbell 1982).

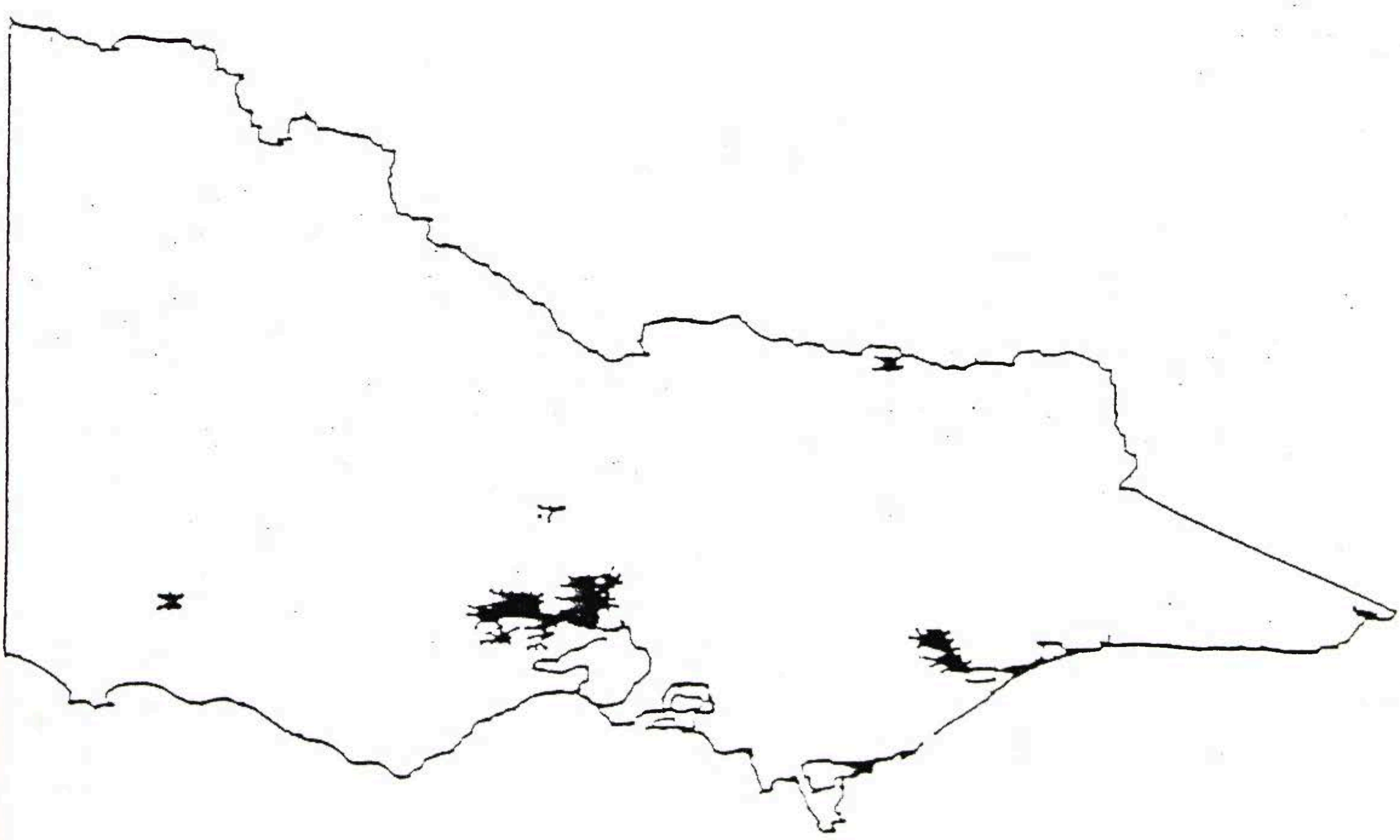


Figure 5: The extent of the invasion by *Nassella trichotoma* in the Western Basalt Plains Grassland (Dainton 1994).

By producing large numbers of seeds, *N. trichotoma* is able to distribute itself widely. ~~Where~~ it now grows in native grasslands or pastoral grasslands. Serrated ~~Tussock~~ becomes the dominant grass ~~as~~ in ~~these~~ such areas stock graze selectively and will not eat *N. trichotoma* unless all other fodder has been removed, consequently Beggs and Leonard (1959) found that sheep on a diet of *N. trichotoma* lost weight and started to die after 7 weeks of grazing (Campbell 1982). This is a result of the relative indigestibility of *N. trichotoma* and its low nutritional value (Campbell 1982; Wilson & Hodgkinson 1990; Dainton 1994). The reduction in the carrying capacity of the land due to Serrated Tussock infestation ~~is causing a~~ can lead to serious ~~loss~~ reduction in farm productivity in areas where it has become a major weed (figures 4 & 5).

Not cited

How do you start to die??

These figs have nothing to do with farm productivity.

~~Thus~~, *N. trichotoma* is not only a problem in the native grasslands but on local farms as well where it outcompetes the pastoral grasses but is unpalatable to stock.

You cannot start para's this way!! repetition

Locally various conservation groups and agricultural interests are concerned with the extensive problem that *N. trichotoma* represents. In 1994 the Victorian Serrated Tussock Task Force, based in Melton, was formed to organise action against the weed.

It has been suggested that periodic fires which are often used to preserve native grassland diversity could also assist the establishment and/or the spread of *N. trichotoma* as well (Lunt 1991; Craigie & Stuwe 1992).

1.5 NUTRIENT DYNAMICS OF GRASSLANDS

In grassland ecosystems the availability of nutrients and soil moisture have been found to be the two consistently major factors that limit the growth of plants. Two nutrients of high significance as limiting factors in many ecosystems, including grassland ecosystems, are nitrogen and phosphorus (McLendon & Redente 1991). There have been few rigorous investigations of the environmental factors limiting the growth of plants in Basalt Plains grasslands. ref

If there have been a few what are they

~~However~~ The cracking clay soil of these areas indicates that soil moisture is one of the major factors, while the low fertility of Australian soils indicates that nutrient levels may be another major factor. refs

Few detailed studies have been published of nutrient dynamics in grassland ecosystems in Australia. Studies that have been undertaken here and overseas give a variety of opinions. For example Culvenor (1981) states that phosphorus is the limiting factor in Australia, while Groves in Australia (1981) and Tilman (1994) in the United States (1994) say that it is nitrogen. Confusing Unclear Not cited

The nutrient levels of Australian soils are generally low when compared to those of other countries. This has been attributed to the geological age of the land (Culvenor 1981). The low nutrient level of Australian soils has resulted in the suggestion that many of the indigenous grasses are adapted to low levels of nutrients in the soil (Culvenor 1981; Lunt 1991).

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; Culvenor 1981). In one of the few controlled experiments in Australia that looked at competition between indigenous and invading species for nutrients, Groves, Keraitis and Moore (1972) examined the relative growth of *Themeda triandra* & *Poa labillardieri* in pots in response to varying phosphorus and nitrogen levels. Although *P. labillardieri* is not an exotic plant it is considered an environmental weed as it invades degraded land. These studies found that when *T. triandra* & *P. labillardieri* were grown together the *Poa* utilised the nutrients much more effectively and dominated the *T. triandra.* pots in response ??

Extensive anecdotal observations and the few rigorous studies available suggest that the non - indigenous grasses often have a competitive edge over the native grasses when nutrients are applied through the direct application of fertilisers, herbicides and chemical fire retardants (Lunt 1991; Craigie & Stuwe 1992) or via the wastes and carcasses of stock. Addition of nutrients can result in a dense growth of exotics which may smother or otherwise out-compete the native species. The growth of exotic species may increase even more if the nutrients are applied to soil that is already disturbed. This response would explain the degradation of native grasslands or farmland, caused by the addition of phosphorus and nitrogen based fertilisers now a common place agricultural practice (Lunt 1991).

1.6 NUTRIENT HYPOTHESIS FOR DISTURBANCE BOUNDARY

At the grassland being investigated in this study (Victoria University of Technology - St Albans campus see section 3.1) there is a distinctive boundary between the *Themeda triandra* and the *Nassella trichotoma*. The side of the boundary on which the *N.trichotoma* is found was ploughed once, many years ago. When even aprox.

This interface between the native grass and the weeds has remained stable for many years and was the starting point for these investigations. why???

One proposed explanation for the influx of weeds onto a site after a soil disturbance (T. McLendon, Dep. Range Science, University of Colorado, pers.comm. to C. Hocking) is that extensive disturbance such as ploughing kills native grassland plants, including their below-ground biomass. This results in the release of nutrients into the soil via microbial degradation. The sudden increase in nutrient levels advantages the growth of weeds, such as *N. trichotoma* which are able to out compete *T. triandra*. Weeds that establish on disturbed sites are those which outgrow natives on nutrient enriched soil and are ultimately able to exclude native plants via a variety of competitive mechanisms (i.e. competition for light, nutrients, moisture, physical crowding).

Mclendon 19?? pers.comm.

1.7 SOIL MOISTURE

Soil moisture is one other major factor that limits the growth of plants in native grasslands (Hagon 1977).

Not cited

Native grasses are generally considered to be better adapted to lower moisture levels than many of the introduced species of grass (Lodder, Groves & Wittmark 1986). Not cited Few studies seem to have been conducted to look specifically at the moisture content of soils supporting native grasses as opposed to introduced species. In one of the few local studies published, Groves (1965) observed the seasonal variation in the soil moisture of a remnant grassland in another area of St. Albans. The soil moisture varied between c. 10 % in summer to c. 30 % in Autumn. In this study he looked only at the growth of *Themeda triandra*.

You should avoid gender

seem to be or have been?? It is not good enough to say seem to be!! You must be direct. Did you fully address the literature??

2. FOCUS OF THE STUDY

The general aim of this study was to investigate what environmental factors are important to grassland ecology and might advantage *Nassella trichotoma* in invading sites with disturbed and undisturbed soil at the expense of *Themeda triandra*; specifically:

1/ To investigate the soil moisture and nutrient content of soil in disturbed and undisturbed sites under *N. trichotoma* and *T. triandra*.

2/ To gather information as to potential expalinations for why *T. triandra* is not easily re-established after soil disturbance.

3/ To establish a general range for the soil nutrient levels (nitrogen and phosphorus) of Basalt Plains Grassland remnants.

3. METHODS

3.1 STUDY SITE

The area of Western Basalt Plains native grassland that was used as the sample site in this study is situated in the south - east corner of the St. Albans campus of the Victorian University of Technology (see figure 6).

The site consists of an area of remnant *Themeda triandra* (Kangaroo Grass) dominated native grasslands. Adjoining this is a heavily degraded section of land that is dominated by *Nassella trichotoma* (Serrated Tussock). This persistant disturbance boundary between *Themeda triandra* and *Nassella trichotoma* was the starting point for these investigations.

A fire went through part of the site in January of 1993. The fire crossed both the remnant and degraded areas of grassland and left mature stands of each remaining. This provided us with the sites that were used for the soil moisture and nutrient studies.

Explain here what it is.

Atrazine was included in the study to compare with a site from which all vegetation had been removed. This site was sprayed two years previously with the herbicide Atrazine.

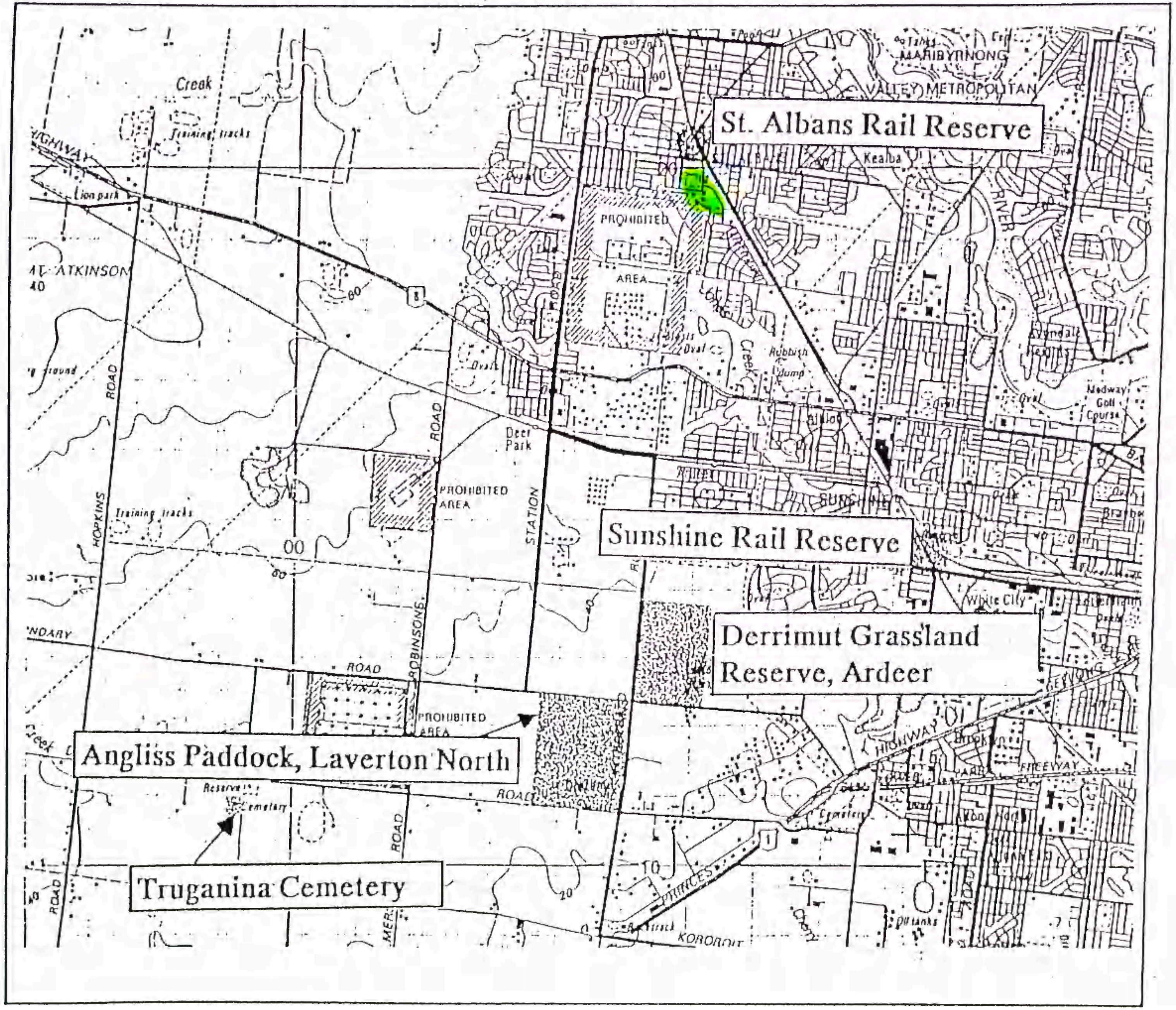


Figure 6: Map showing the location of the study site in relation to other native grassland reserves in the western suburbs of Melbourne (McDougall & Kirkpatric 1994). — Not cited

3.2 ANALYTICAL METHODS FOR NUTRIENT ANALYSIS OF SOIL AND PLANT MATERIAL

The methods used to determine the amounts of phosphorus and nitrogen present in the soil and plant samples are outlined below.

3.2.1 ANALYSIS OF TOTAL PHOSPHORUS

Two methods were employed in this study. The first was the Injection Flow Analysis method. When it was clear that this-was unsuitable for phosphorus (P) analysis in Basaltic clay soils the Spectrophotometric method was used.

The analysis of phosphorus in plants and soil samples using both methods involves the ashing of the substance being sampled. The steps shown below are based on Piper (1944). The ashing procedure was also used to estimate the residual root content of the dried soil samples (see section 4.2.2).

1/ A dry crucible is oven dried at a temperature of 105°C for at least half an hour to remove any moisture.

2/ The crucible is placed in a desicator until cool and weighed to $\pm$ 0.0001g

3/ The sample is weighed, then left to dry overnight at 105°C

4/ This is then placed in a desiccator and weighed when cool.

5/ The sample is placed in a muffle furnace overnight at 420°C. The temperature of the muffle furnace should be raised slowely to 420$\pm$5°C. The resulting soil should be red or orange in colour.

6/ The crucible and the contents are placed in a desiccator and when cool are weighed to obtain the weight of the crude ash.

7/ The crucible is covered with a watch glass and the ash is moistened with 4 - 5 drops of distilled water. Then 3 ml of 5M hydrochloric acid is carefully pipetted under the lip of the watch glass. The crucible is covered with a watch glass so as to prevent loss by effervesence.

8/ The crucible is placed on a boiling water bath and digested for 2 hours.

9/ The cover of the crucible is removed and rinsed and 0.2ml of 15M nitric acid added. Evaporate the solution until dry.

10/ The crucible is placed on a hot plate for at least 1hr or until totally dehydrated.

11/ The dry salts should be moistened with 2ml of 5M hydrochloric acid. 10ml of distilled water is added and warmed on a water bath for approximately 30 minutes.

12/ The solution is placed in a 250ml volumetric flask and diluted to volume with distilled water.

13/ Using a centrifuge, spin the samples to separate the solids from the solution.

14/ Transfer to a 250ml polyethylene bottle and add a crystal of thymol to preserve the solution.

ESTIMATE OF TOTAL PHOSPHORUS USING INJECTION FLOW ANALYSIS:

To determine the total phosphorus content of this solution the original method used was by injection flow analysis using the Tector Aquatec. This involves the reaction of orthophosphate in the sample with ammonium molybdate to form heteropoly molybdophosphoric acid. This is then reduced by stannous chloride in a sulphuric acid medium, to form phosphomolybdenum blue which is measured spectrophotometrically (Tecator 1990).

Not cited

1/ 15.0 ml of solution is placed in a n autoclave proof glass bottle.

2/ 0.15 ml of 4M sulphuric acid and 3.0 ml of the potassium persulphate (appendix 1) is added.

3/ This is heated for 30 minutes in an autoclave, and analysed when cool.

This method produced a yellow/orange suspension in the solutions which would not redisolve. These particulates caused problems by clogging tubes in the flow analysis system and could therefore not be analysed. Filtering the solutions was not an option at this point as samples analysed before clogging showed that the phosphorus was actually bound in the precipitate, with only small amounts in the solution.

THE SPECTROPHTOMETRIC METHOD TO ESTIMATE TOTAL PHOSPHORUS:

Following the failure of the above method a second was used. This involved determining the concentration of phosphorus in an aliquot of the solution prepared by the above ashing method using a spectrophotometer and standard curve.

Not cited

The spectrophotometric method for the preparation of aliquots shown below is based upon Murphy and Riley, 1962. This involves the reduction of a phosphomolybdate complex by ascorbic acid using an antimony catylist.

1/ For preparation of reagents, refer to appendix 1.

2/ Standard curves were prepared in the concentration range expected for phosphorus in the samples.

3/ The acidity of the samples was checked and adjusted to an approximate pH of 3 by the addition of sulfuric acid or sodium hydroxide.

4/ Distilled water is then added to adjust the volume to 45 mL, and the solution is mixed.

5/ Five mL of reagent B (see appendix 1) is added and the solution made to volume.

6/ The solution was then heated in a constant temperature water bath at 70°C for 10 minutes.

Type

7/ When cool, the absorbance of the solution can be determined by a spectrophotometer at 880*mu*. Samples should be read against a reagent blank prepared with distilled water. The solution must be analysed as soon as possible since it is only stable for 8 hours.

3.2.2 ANALYSIS OF TOTAL NITROGEN TKN.

The method for the analysis of Kjeldahl nitrogen in the soil samples is based on Rump & Krist (1988). The process is as follows:

1/ Approximately 1 - 2g of dry soil is placed in a Kjeldahl tube.

2/ 1g of reaction mixture (see appendix 2) is added to the soil sample as well as 10ml of ethanol.

3/ After shaking, 10ml of concentrated sulfuric acid is carefully added. The solution is heated slowly and boiled for 30 minutes.

4/ After cooling, the digest is placed in a one litre distillation flask and made up to 300mL with distilled water, rinsing the Kjeldahl tube.

5/ Add a few drops of phenolphthalein solution with sufficient sodium hydroxide to colour it pink (approximately 40 mL).

6/ The flask is attached to distillation apparatus and distilled to a known volume (approximately 200ml) distils over. During this time the end of the condenser should dip into the absorber.

Initially a titrimetric method was used for the analysis of Kjeldahl Nitrogen but this method proved too insensitve to determine the concentrations encountered.

ESTIMATION OF TOTAL KJELDAHL NITROGEN USING INJECTION FLOW ANALYSIS

The determination of the Total Kjeldahl Nitrogen (TKN) involves the use of the Tecator Aquatec by injection flow analysis (Tecator 1991). This involves the distillate being injected into the Aquatec where it combines with aqueous sodium hydroxide. Ammonia is formed which diffuses through a gas permeable membrane into an indicator stream which consists of a mixture of acid-base indicators that will react with the Ammonia. This results in a colour change which is measured photometrically. The reagents and standards used are shown in Appendix 2.

Problems with this method were encounted because of the rapid volatization of ammonia which meant that sample solutions needed to have their pH lowered to stabilize the solutions. This caused the flow analysis system to give inaccurate values regarding the TKN concentrations of the solutions, as the method relies on changing the pH just prior to photometric determination by the addition of sodium hydroxide into the analysis stream. It was found after some investigation that using the Tecator Aquatic that each sample had to have its pH readjusted before analysis. The need for pH readjustment of acid stabilised samples was only discovered in the course of analysis. This procedure was not clear in the instructions for the Aquatec. As a result, the reliability of some early TKN data was rendered questionable.

Another problem with this method was the instability of the base line on the flow analysis system, and the sensitivity of the indicator which needed continual readjustments.

The third problem with the flow analysis system using the Tecator Aquatec was the unreliability of the machine, it continually developed leaks and other related problems.

3.3. METHOD FOR THE DETERMINATION OF SOIL MOISTURE

When taking soil samples from the grasslands to look specifically at gravimetric analysis of soil moisture, two methodological problems presented themselves.

1/ The potential loss of moisture during transfer of samples between the grasslands and the laboratory.

2/ The error introduced with

a. the presence of rocks, which take up space and weight but hold little or no water. Their presence would therefore affect the % moisture calculations.

b. or large amounts of living root material which would hold moisture which was not part of the soil moisture, but would contribute to gravemetric estimation of soil moisture.

In order to minimise any potential loss of moisture and to take into account the presence of confusing factors (rocks and roots) the procedure below was developed:

1/ Labels were placed on empty samples vials which were oven dried with their lids at approximately 60°C.

- The combined weight of each vial and its lid is recorded on the vial.

2/ Core samples were collected from depths of 1-4, 5-8 and 15-18 cm to obtain a depth profile of soil moisture. The lids were affixed tightly.

3/ Immediately upon return to the laboratory the vials were weighed to obtain the combined weight of the vial and the core sample

- From this the fresh mass of the soil was determined. The fresh mass included the soil, rocks, roots and moisture. The samples were stored in the cold room until they were processed.

4/ Cores were removed from vials and the rocks and roots carefully separated from the soil. The fresh weight of the roots is determined immediately.

5/ The rocks were returned to the vial and a small amount deionised water is added to wash off any soil or rocks still in the vial. This water (minus the rocks) is added to the soil sample.

6/ For each sample the soil, roots and rocks dried separately and the weights recorded.

7/ To calculate the moisture content of each core sample ;

a/ The fresh weight of roots and the dried weight of rocks is subtracted from fresh core weight. This will give the fresh weight of the soil.

b/ The loss of moisture due to drying is determined by subtracting the weight of the dry soil from the weight of the wet soil (determined above).

c/ The loss of moisture is expressed as a percentage of the dry weight of the soil.

4. RESULTS AND DISCUSSION

4.1. NUTRIENT DETERMINATION

INITIAL ANALYSIS OF TOTAL KJELDAHL NITROGEN

Initial studies conducted for the determination of the nitrogen and phosphorus content of the soil of a *Themeda* site and a *Nassella* site are shown in figures 7 & 8. Figure 7 shows the results of the analysis for Total Kjeldahl Nitrogen (TKN). This figure does not give any immediate indication of a significant difference in TKN values of either site. Statistics conducted using the two-way analysis of variance showed that there was no statistically significant difference between the TKN concentrations of the *Themeda* and the *Nassella* sites.

INITIAL ANALYSIS OF TOTAL PHOSPHORUS

The data obtained for the initial analysis of the total phosphorus concentrations of the two sites is shown in figure 8. This data was not determined to be statistically significant using a two-way analysis of variance test. Results of ANOVA ??

FOLLOW UP STUDY

A second round of sampling was done to reexamine the nutrient values obtained between the different sites. These are shown in figures 9, 10 & 11). Neither the Total Kjeldahl Nitrogen or the Total Phosphorus concentrations were found to have a statistically significant difference in values. Results of statistics??

Neither of these studies showed any significant difference in the Total Kjeldahl Nitrogen or the Total Phosphorus concentrations between any of the sites.

The lack of a statistically significant difference in the nutrients concentrations between the sites does not necessarily mean that nutrients levels do not play a role in the establishment of introduced species. These nutrient levels could be a short term effect of other factors. There is an almost complete lack of data on the role that nitrogen and phosphorus play in the Basalt plains grasslands.

There is some doubt regarding the accuracy of the values that were obtained for the nutrients in this study. This is due to the methods used for the determination of the nutrients and the problems associated with them. Any nutrient results would need further verification before being considered reliable.

While these samples were being analysed for their nutrient content, the soil moisture was also determined by the simple method of oven drying overnight. The results of this initial soil moisture determination showed a difference in the soil moisture between the burnt and the unburnt *Themeda* sites which formed the basis of the second factor being looked at - soil moisture. These results were followed up in later studies.

You have not referred to figures

Refs ?

FIGURE 7: Average Total Kjeldahl Nitrogen determined during initial sampling (ppm).
Error bars represent standard deviation, here and on all other graphs.

Why? what about S.E.

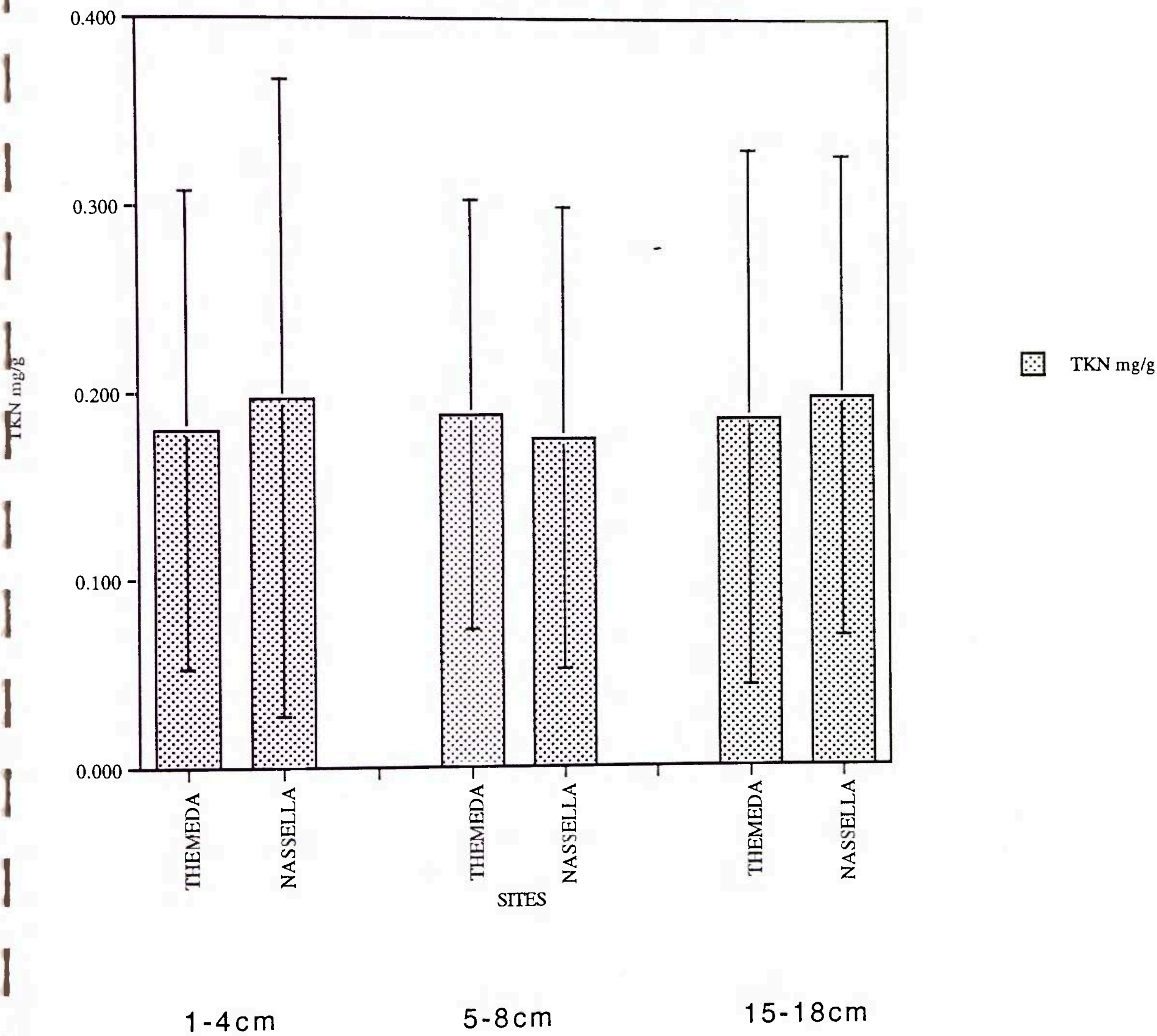
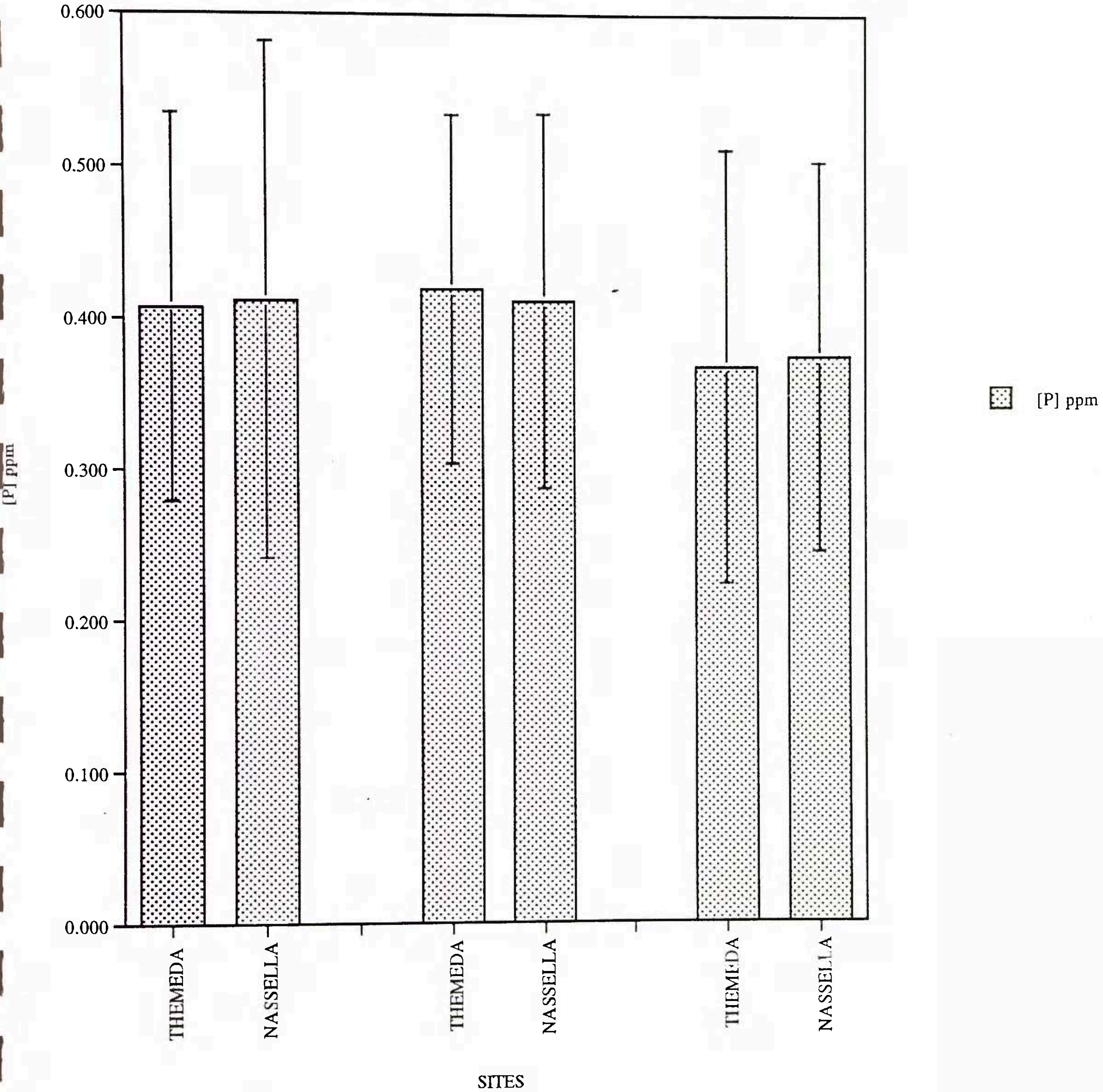


FIGURE 8: Average Total Phosphorus determined during initial sampling (ppm). Standard deviation included. « S.E. ??



1-4 cm 5-8 cm 15-18 cm

FIGURE 9: Average Total Kjeldahl Nitrogen determined in follow up nutrient sampling. Standard deviation included.

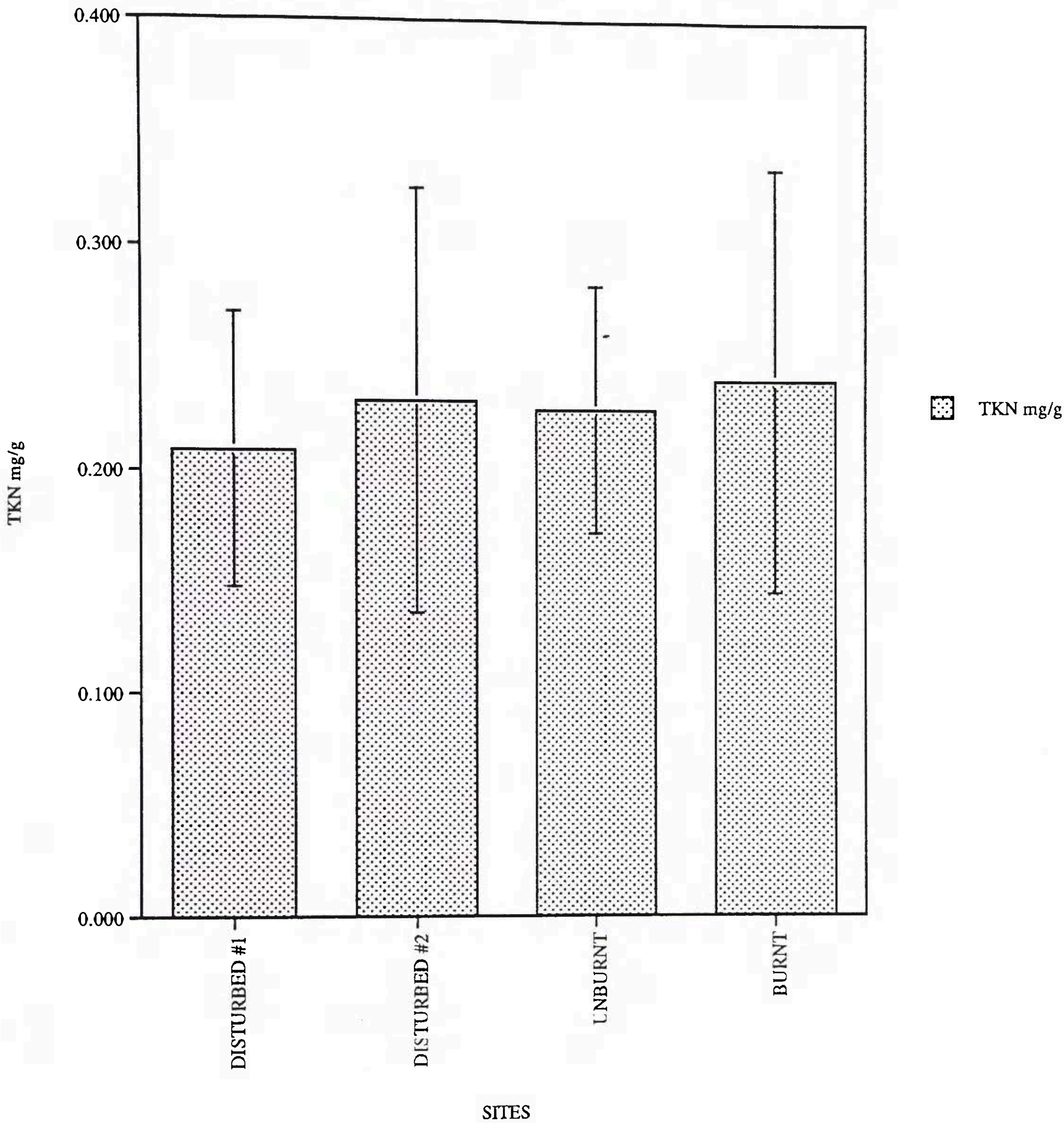


FIGURE 10: Average Total Phosphorus determined in follow up nutrient sampling. Standard deviation included.

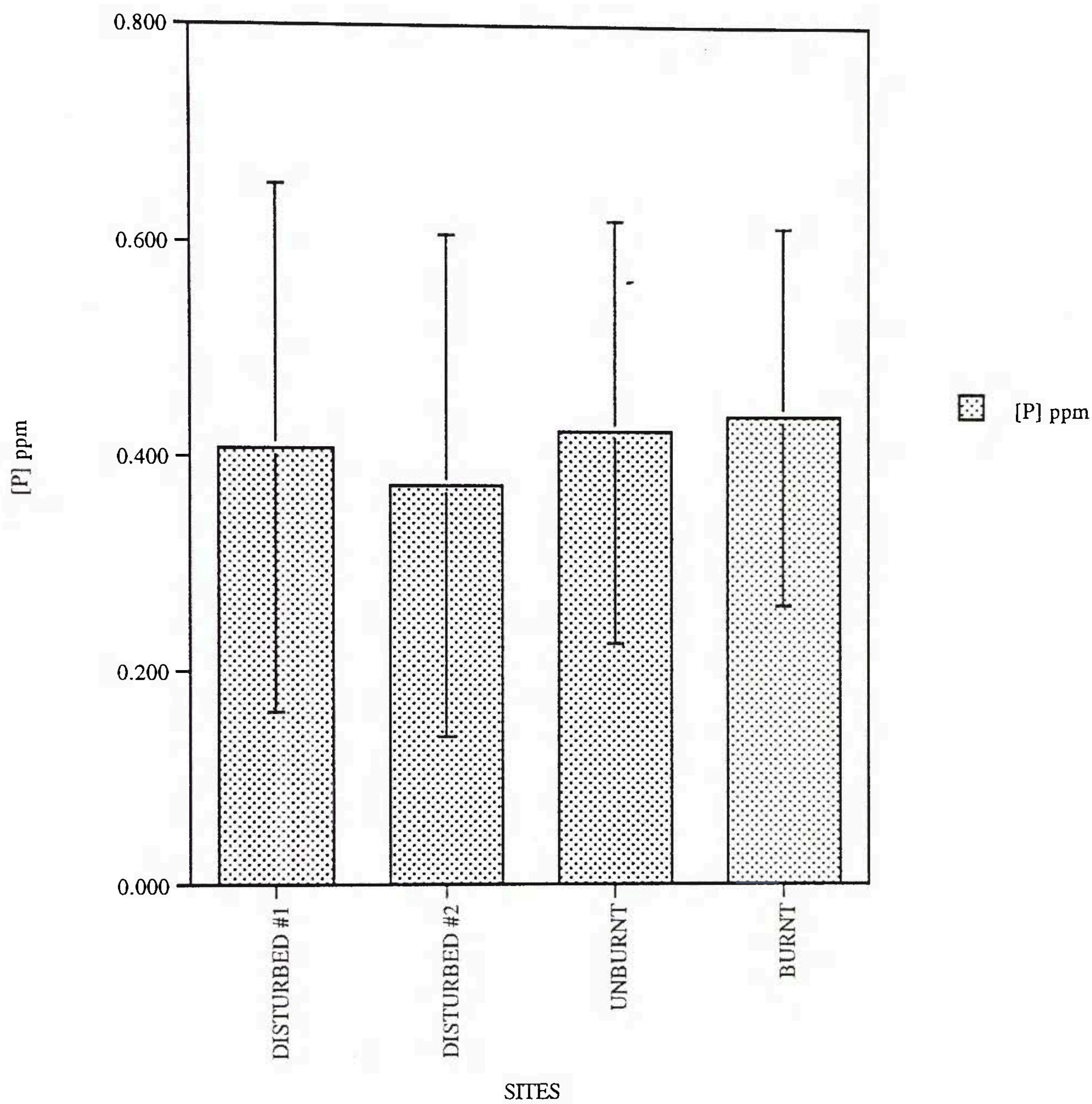
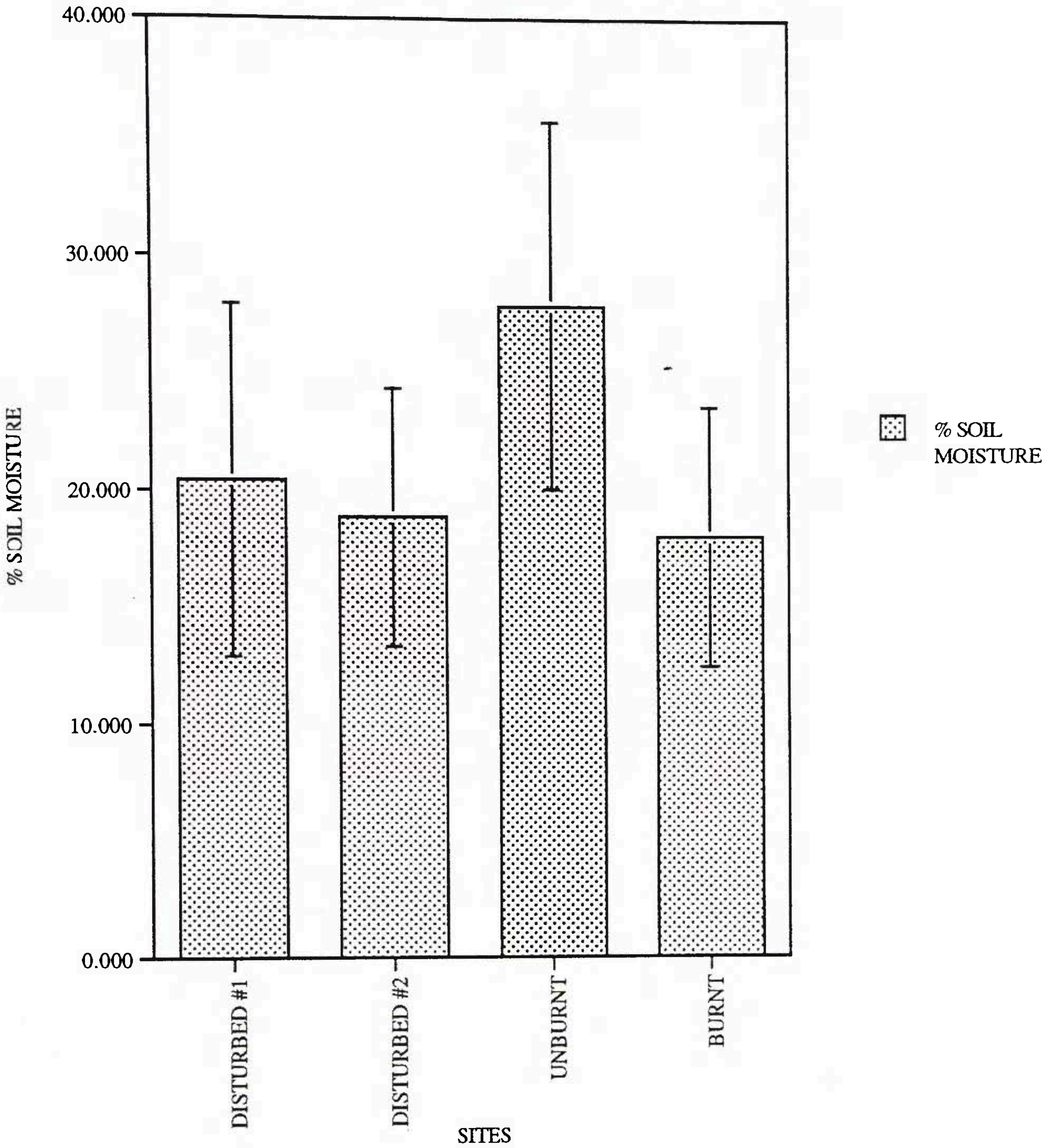


FIGURE 11: Average soil moisture content determined during nutrient investigations.



4.2. SOIL MOISTURE

4.2.1. RANGE FINDING STUDY

PRELIMINARY FINDINGS

The initial range finding test conducted during a dry period of winter showed a substantial difference in soil moisture between the unburnt *Themeda* site and the unburnt *Nassella* site (figure12), with the unburnt *Themeda* obviously having a much higher soil moisture content than the unburnt *Nassella*. This was also the case for the burnt *Themeda* site. This was observed at all 3 levels that samples were taken at.

Also observed was the fact that the moisture content for the *Nassella* dominated sites was generally closer to that of the bare ground (Atrazine treated) site, from which most vegetation had been removed years ago.

This suggested that perhaps the mature stands of *Nassella* had the effect of significantly lowering the soil moisture content of the soils from their previous values.

~~what about stats~~

STATISTICAL ANALYSIS OF DATA

Initial statistics conducted on these result are shown in tables 1, 2, & 3. These were obtained using Minitab to conduct a two-way analysis of variance, as a three-way test was not available. All data underwent an Arcsine transformation to normalise it. Statistical significance was taken at the 0.05 level. When statistical significance has been determined it was always showing that the soil moisture under the *Nassella* was lower than that of the *Themeda* sites, and that the burnt sites had lower values than the unburnt. Additional statistics were conducted for all different studies using the Mann-Whitney (non-parametric) test, but none of the data collected in the initial study was found to be significant by this method as too few replicates were taken (3 only).

what about power?? why were 3 only taken. what about design??

Systat

what table

The two-way analysis of variance (table) shows that the only statically significant difference in soil moisture values in this limited study were obtained in the 1-4 cm layer, and occured due to the different species of grass growing on the soil. With the *Nassella* sites having significantly lower values than the *Themeda*. At the 5-8 cm level there was no statistically significant differences in values. The species of grass growing was also found to be statistically significant at in 15-18 cm layer, and here, the interaction between the two factors was found to be significant.

are you sure

How do you know it is the grass causing lower the moisture

There can be an interaction but that does not relate to cause + effect !!

Fire history was not determined to be significant for this initial study even though the interaction of it with the species was. This could be due to the small number of replicates taken (tables 1, 2, & 3).

What about design

After the initial study, problems with the method for the determination of soil moisture were addressed - section 3.3.

4.2.2. DETAILED FOLLOW UP OF MOISTURE UNDER *THEMEDA* AND *NASSELLA*

Detailed sampling of soil moisture was undertaken to follow up the initial study. This occured after a day of fairly heavy rain which might account for the raise in soil moisture levels at the surface, above those initially determined.

Probably !!

The data followed the same general pattern of the range-finding test, with the unburnt *Themeda* site being substantially higher in soil moisture than the unburnt *Nassella*, or infact any of the other sites (1-4cm). Soil at 1-4 cm under the burnt *Themeda* also had a substantially higher value than the unburnt *Nassella*. The burnt *Nassella* had an average soil moisture value of between the burnt and unburnt *Themeda*.

Statistic s conducted on this study by a two-way analysis of variance (tables 4, 5, & showed that there was a statistically significant difference in the soil moisture in the 1-4 cm layer, with the sites recently burnt being lower in soil moisture.

Statistical analysis conducted using the Mann-Whitney test showed a statistically significant difference between the two *Themeda* sites with different fire histories, but no difference between the *Nassella* sites. Significance

Two-way analysis of variance also showed a highly significant effect at this level to the two different grasses (*Themeda triandra* and *Nassella trichotoma*) growing on the site.

For the soil moisture of the two different grasses growing on the unburnt site, the Mann-Whitney test determined that there was a highly significant difference. This was not the case for the recently burnt site.

The interaction of these two factors, given by the two-way analysis of variance was also higly significant and showed that these factors contribute to the difference in soil moistures between sites. Contribute or inter^n !!??

At the 5-8 cm and the 15-18 cm levels the fire history was not determined to be statistically significant using the two-way analysis of variance, and the only statistically significant difference determined by the Mann-Whitney test was in the 5-8 cm level.

These samples did show statistical significance regarding diiferences in the soil moisture between the two different species. At both levels the combined effect of fire history and species present produced a statistically significant result using both the two-way analysis of variance and the Mann-Whitney test.

EFFECTS OF ROOTS ON SOIL MOISTURE

As part of this study the effects of roots in the soil samples on the soil moisture determination using the gravimetric method were investigated. These are discussed below.

ROOT REMOVAL EFFICENCY

Ashing was used to estimate the carbon content of the samples, this would consist of the roots and other plant material and microbial biomass. Ashing would therefore give us the upper limit estimation of the residual root content in the soil samples for which root removal has been undertaken.

Figure15shows the average weight lost from the soil samples after ashing, as a percentage of the dry soil weight before. This information was used to help determine the efficency of the root removal from the soil samples. The actual removal of roots was as complete a job as reasonably possible.

What does this mean? You cannott say this!!

The lowest values obtained throughout the entire study are positined on the left side of the graph. These were compared to the average of the five highest values from each different site. In general the root removal was no greater than 1% above the minimum values, indicating that a high proportion of roots had been removed. The Atrazine site lost a much higher proportion of its weight during ashing than any of the other sites. The high carbon content of this site (as determined by ashing) is unlikely to be due to residus root material, little root was found in soil samples from this site. It is possible that the high carbon content is due to a high microflora in the soil, present because of degraded root matter.

refs

ROOT MOISTURE AS A PROPORTION OF SOIL MOISTURE.

Figure16hows the average values obtained for the root moisture as a proportion of the soil moisture. Root moisture was determined gravimetrically by taking before and after drying weights of root material removed from soil samples. From this graph we can see that the roots in a sample generally contribute less than 1% to the moisture content of a sample. With this in mind the necessity of removing the roots from each sample was generally considered not to be nesessary.

~~However,~~ There was one exception, some of the samples found in the unburnt *Themeda* sites, had large enough amounts of roots present to interfer with accurate soil moisture calculations. Therefore when determining soil moisture gravimetrcally for soil samples taken from mature stands of *Themeda*, the removal of roots prior to gravimetric estimation is advisable.

ROOT MASS AS A PROPORTION OF SOIL MASS

Figure17 shows the proportion of wet soil mass that can be attributed to the roots.

From this graph we can see that the greatest root biomass was found in the 1-4cm layer of samples, as would be expected, and the lowest root biomass in the 15-18cm level, also as expected.

The Atrazine sight had the lowest root biomass throughout all the layers. This would also be expected as there has been little or no vegetation on the site since it was sprayed in approximately January 1993.

The unburnt *Nassella* seems to follow Atrazine with the low presence of roots, while the *Themeda*, on both the burnt and unburnt sites, has the greatest biomass, especially the unburnt 1-4cm samples.

4.2.3. SUBSEQUENT SAMPLING

The second round of follow up samples were taken after a couple of hot (~30°C) days and consequentially, the surface soil moisture is substatially lower than either of the other two sample dates.

The trends in soil moisture in these samples followed the other two with the unburnt *Themeda* being significantly higer than any of the other sites, especially the unburnt Nassella.

?

Statistical analysis (table) showed that these samples also had statistically significant differences in the soil moisture of the 1-4 cm layer, caused by the fire history, with the recently burnt sites being lower, but no difference in the 5-8 cm and the 15-18 cm levels. The Mann-Whitney test did determine a difference between the burnt and unburnt *Nassella* sites at the 15-18 cm level.

The species present did have a statistically significant sffect on the soil moisture in the 1-4 cm layer, but ther was no determined difference in the soil moisture between the species at either the 5-8 cm or the 15-18 cm level for these samples. The combined effect of fir history and species present did have a statistically significant effect on the soil moisture.

4.2.4. SUMMARY OF SOIL MOISTURE FINDINGS

For both of the detailed studies looking at the soil moisture between the burnt and unburnt sites of *Nassella* and *Themeda*, the difference found between the lower values of the burnt sites and the higher values of the unburnt sites was found to be statistically significant in the 1-4 cm level. Neither the 5-8 cm or the 15-18 cm levels had a significant difference in soil moisture regarding the fire history for the differnt species for either for either of the follow up studies. This indicates that although the fire might have had a potential effect on the soil moisture of the sites, any significant effect it did have was limited to the first layer i.e. 1-4 cm.

The effect of the grass species growing on the different sites had a statistically significant difference on the soil moisture in the 1-4 cm level of all three sets of studies. The effect on the soil moisture of the 5-8 cm and the 15-18 cm layers was not found to be significant in the initial study or in the last set of samples, though statistical significance was found in the first follow up study at these levels.

The interaction of the twofactors, fire history and species growing was found to be statistically significant at all levels in the two follow up studies, but only in the last level of the initial study. These factors had the effect of lowering the soil moisture in soils from the unburnt *Nassella* stands and raising moisture in the unburnt *Themeda* stands.

Elevation or stage

4.2.5. IMPLICATIONS FOR THE MANAGEMENT OF REMNANT GRASSLANDS

Really? Do you really have evidence for this??

The results obtained here suggest that mature stands of *Nassella* are actively lowering the soil moisture of the grassland soils. This is evidenced by the fact that the stands of mature *Nassella* have significantly lower soil moisture than the recently burnt stands and that the soil moisture in these stands is generally closer to that of the bare ground (Atrazine) site than any of the others.

This is not convincing!!!

The soil moisture has previously been indicated to be one of the major factors effecting plant growth in native grasslands. If the *Nassella* does infact have a significant effect in lowering the soil moisture this would effect *Themeda's* ability to compete with *Nassella* where it has established.

Hagon (1977) showed that for *Themeda* seeds to germinate they need a high soil moisture content. If the soil moisture is actively being lowered by the mature stands of *Nassella* then the *Themeda* has less chance of establishing.

Not cited

Methods need to be considered that would raise the soil moisture levels in *Nassella* dominated soils and allow the establishment and growth of *Themeda*. Thatching is one method which might be considered as it would retain soil moisture by developing conditions similar to mature stands of *Themeda* were there is a dense ground covering which helps retain soil moisture.

Though soil moisture is one of many factors that need to be considered when looking at the re-establishment of *Themeda* on *Nassella* dominated sites it is one that is significant and needs to be taken into account.

FIGURE 12: Average soil moistures taken at 3 levels, for the initial range finding samples. Standard deviation included.

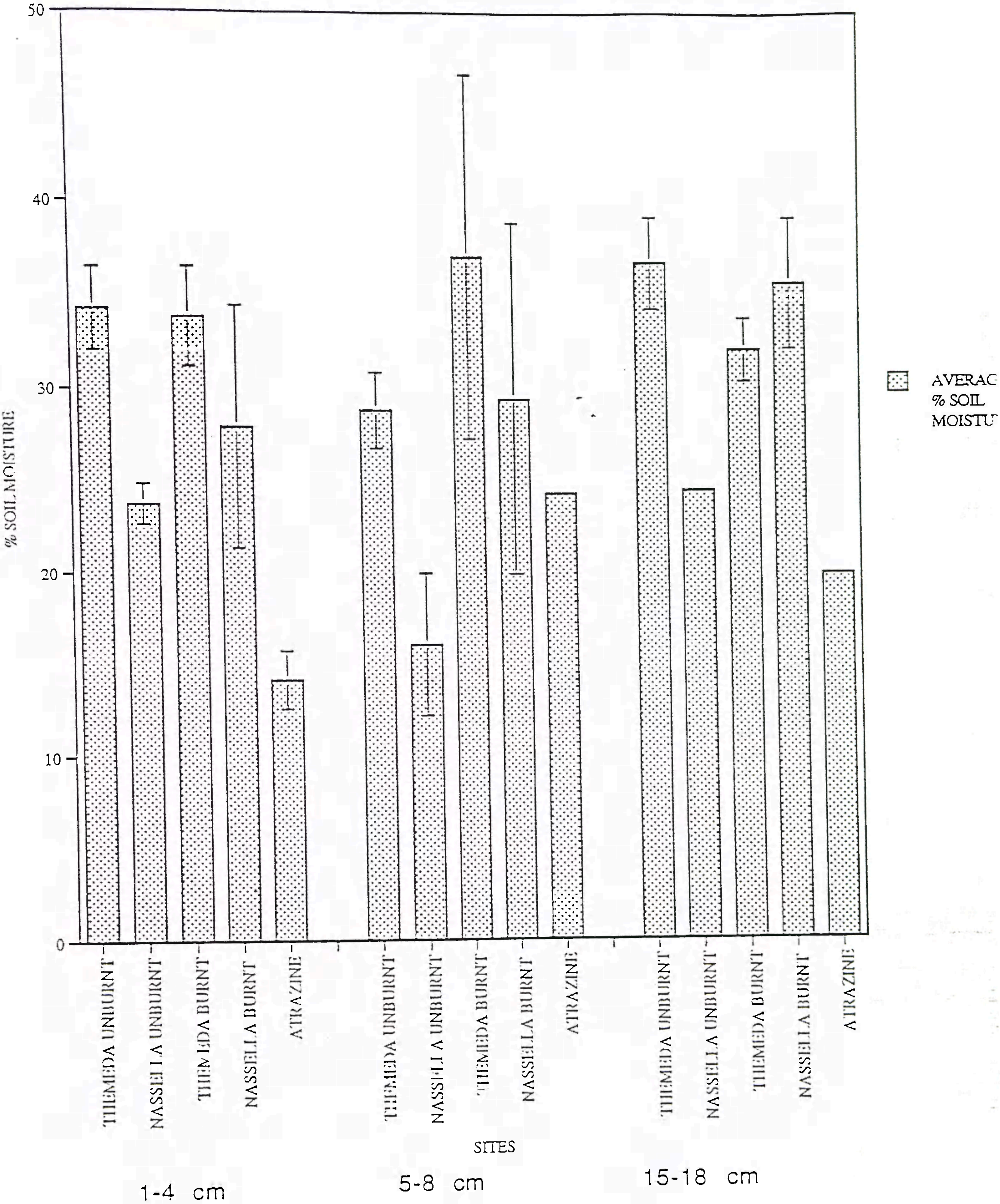


FIGURE 13: Average soil moistures taken at each level for the detailed follow up of soil moisture. Standard deviations included.

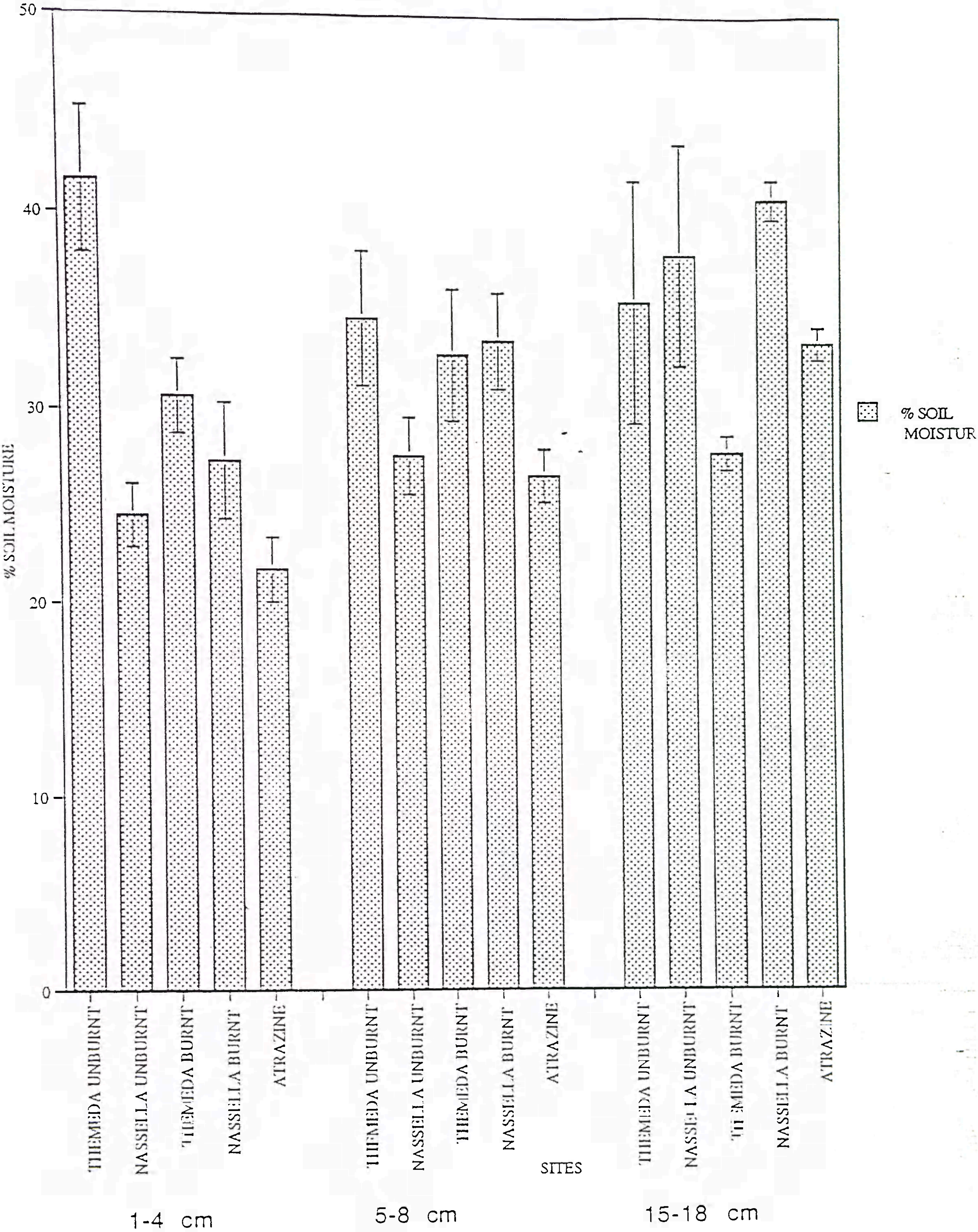


FIGURE 14: Average soil moisture obtained at each level for the final soil moisture study. Standard deviations included.

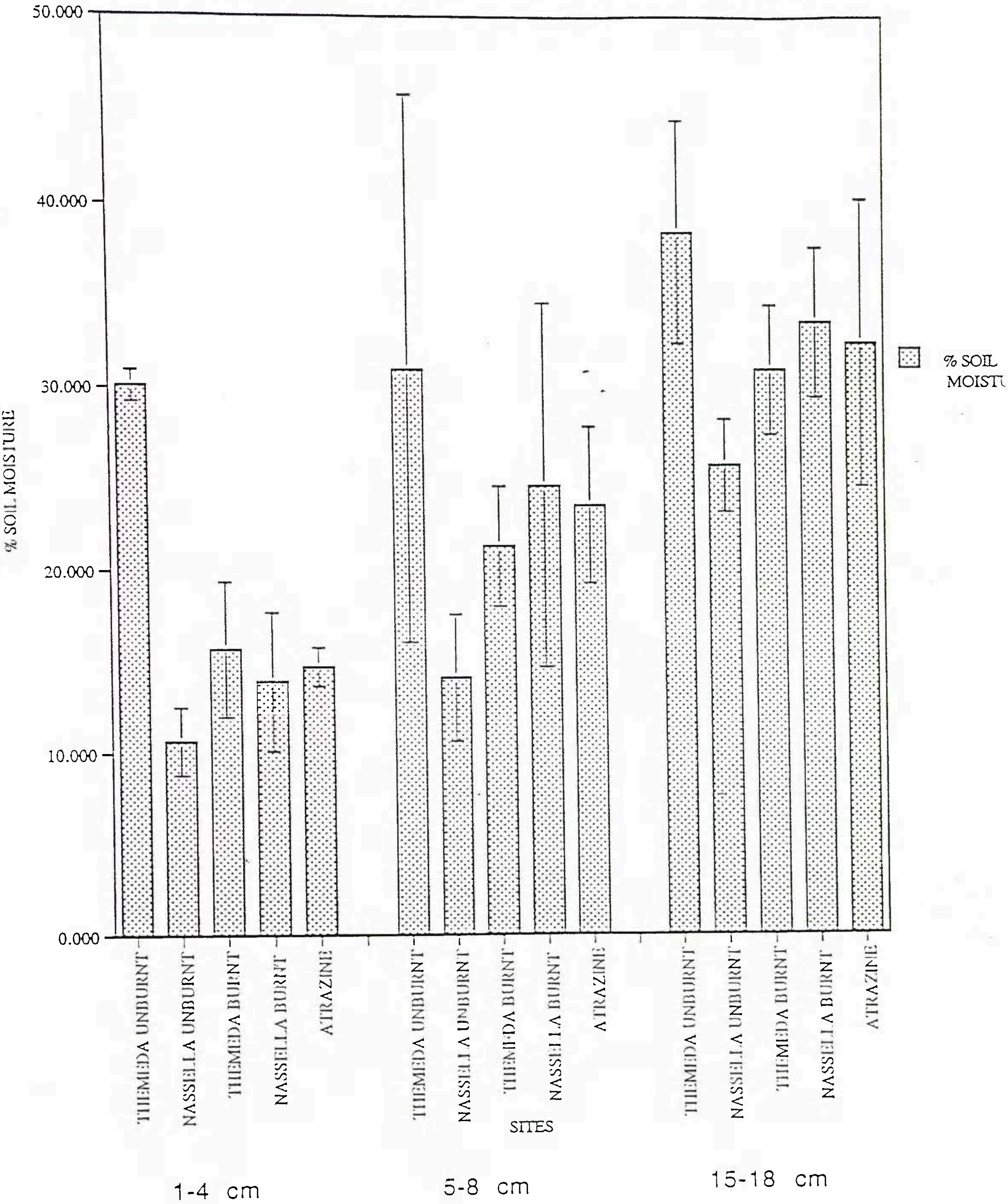


FIGURE 15: Average mass lost during the ashing of samples (%) - root removal efficency. Standard deviation included.

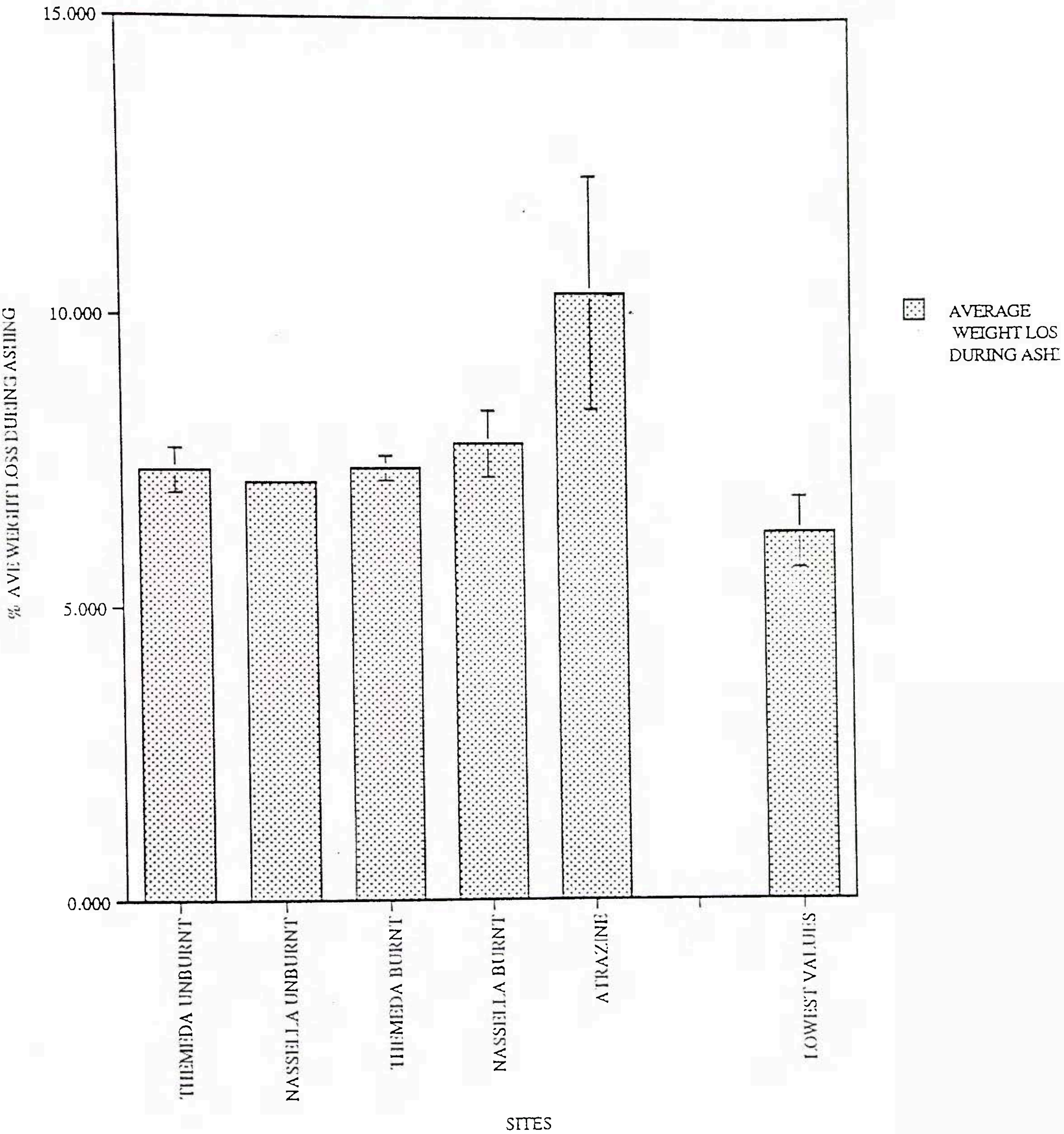


FIGURE 16: Average root moisture as a proportion of the soil moisture.

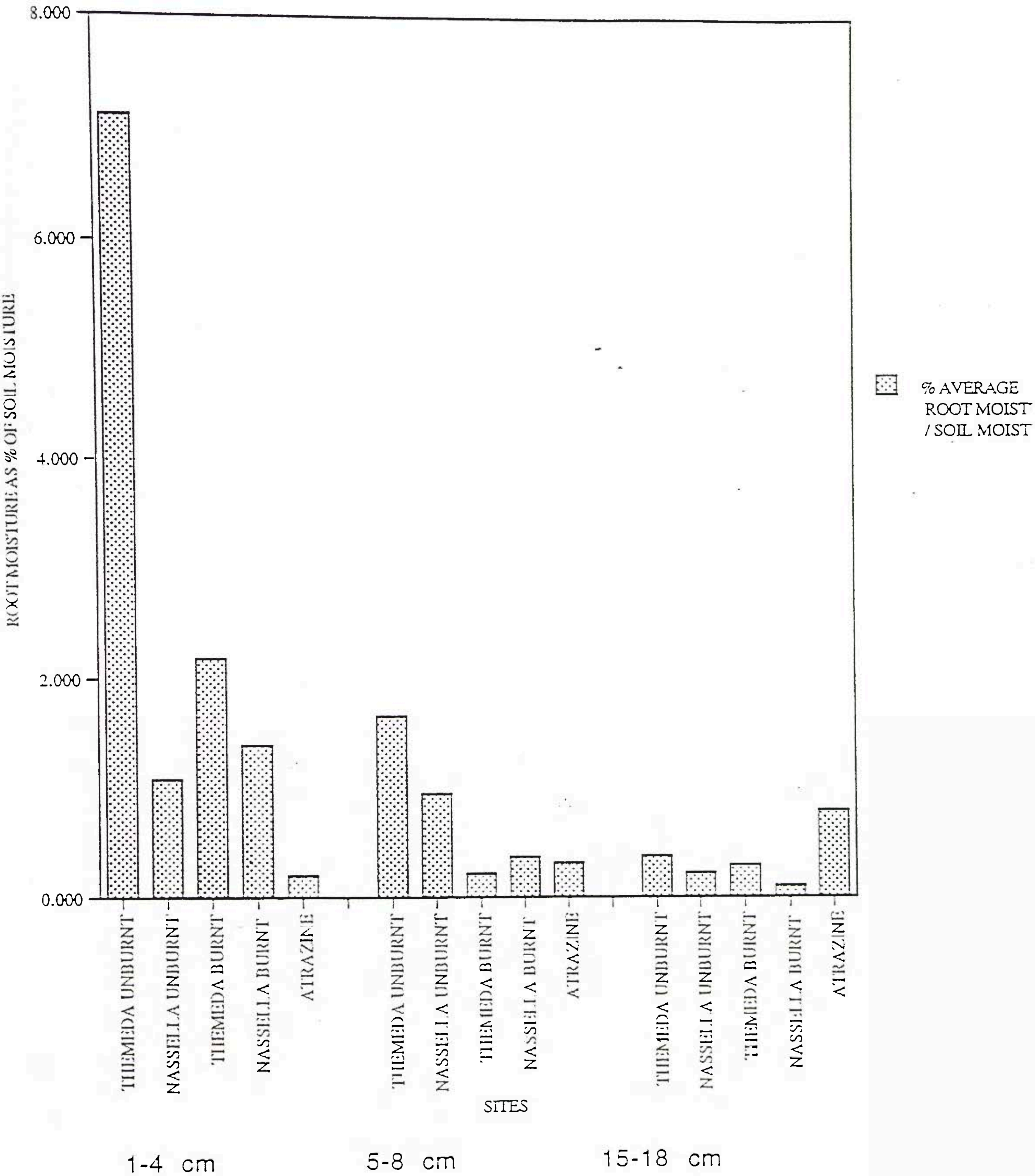


FIGURE 17: Average root mass as a proportion of the soil mass.

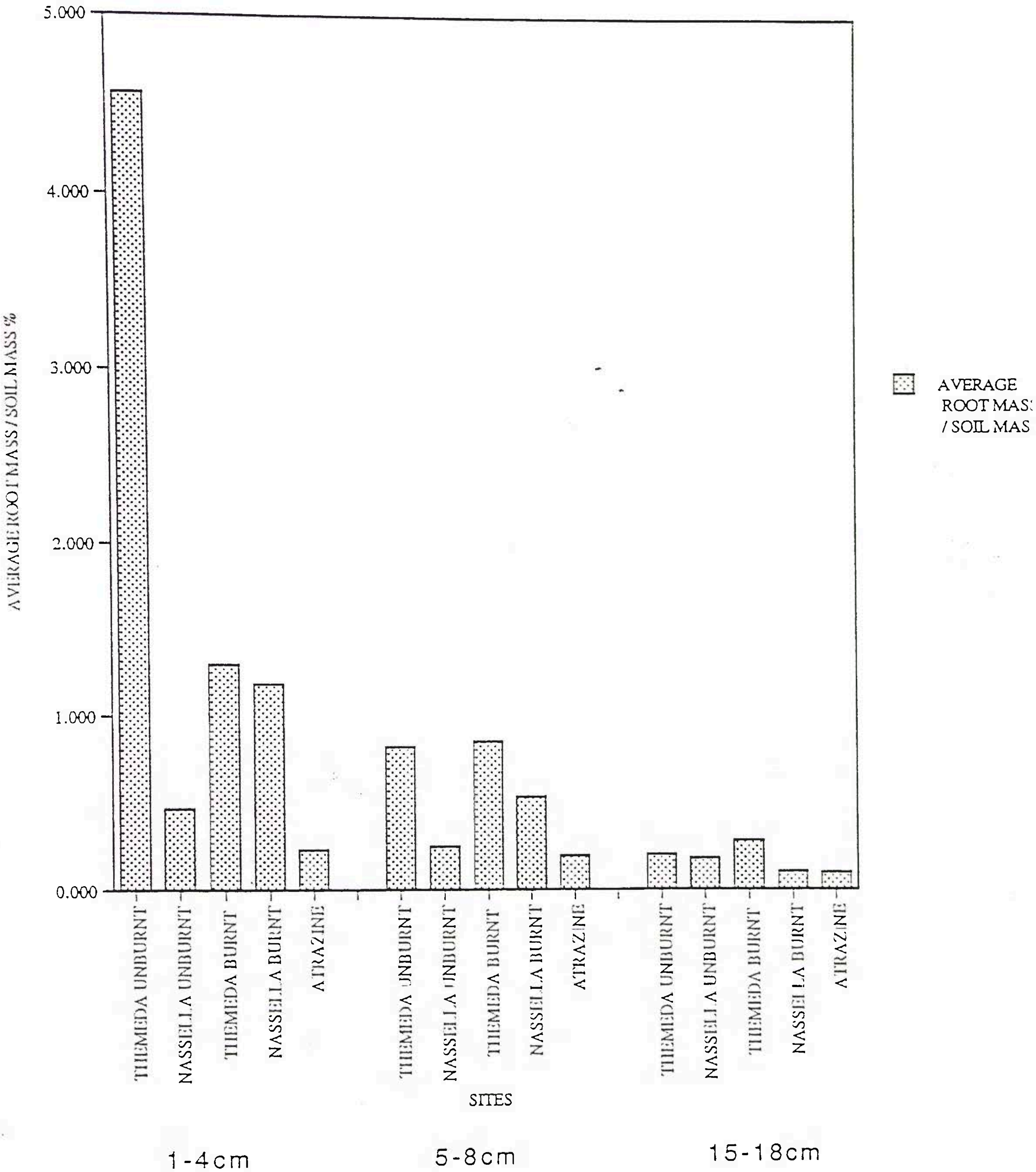


TABLE 1: Determination of statistical significance for the range finding test in the 1-4cm level.

SOURCE OF VARIATION	STATISTICAL SIGNIFICANCE
FIRE HISTORY	$0.25 < P$
SPECIES	$0.025 < P < 0.05$
INTERACTION	$0.10 < P < 0.25$

5.10.94 1 -4 cm

TABLE 2: Determination of statistical significance for the range finding test at the 5-8 cm level.

SOURCE OF VARIATION	STATISTICAL SIGNIFICANCE
FIRE HISTORY	$0.05 < P < 0.10$
SPECIES	$0.05 < P < 0.10$
INTERACTION	$0.25 < P$

5.10.94 5 - 8 cm

TABLE 3: Determination of statistical significance for the range finding samples at 15-18 cm level.

SOURCE OF VARIATION	STATISTICAL SIGNIFICANCE
FIRE HISTORY	$0.05 < P < 0.10$
SPECIES	$0.05 < P < 0.10$
INTERACTION	$0.0025 < P < 0.001$

5.10.94 15 - 18 cm

TABLE 4: Determination of statistical significance for the follow up sampling at the 1-4cm level.

SOURCE OF VARIATION	STATISTICAL SIGNIFICANCE
FIRE HISTORY	$0.0025 < P < 0.005$
SPECIES	$P < 0.0005$
INTERACTION	$P < 0.0005$

1 - 4 cm

TABLE 5: Determination of statistical significance for the follow up sampling at the 5-8cm level.

SOURCE OF VARIATION	STATISTICAL SIGNIFICANCE
FIRE HISTORY	$0.10 < P < 0.25$
SPECIES	$0.025 < P < 0.05$
INTERACTION	$0.01 < P < 0.025$

5 - 8 cm

TABLE 6: Determination of statistical significance for the follow up sampling at the 15-18cm level.

SOURCE OF VARIATION	STATISTICAL SIGNIFICANCE
FIRE HISTORY	$0.25 < P$
SPECIES	$0.001 < P < 0.05$
INTERACTION	$0.01 < P < 0.025$

15 - 18 cm

TABLE 7: Determination of statistical significance for the final sampling at the 1-4cm level.

SOURCE OF VARIATION	STATISTICAL SIGNIFICANCE
FIRE HISTORY	0.0025 < P < 0.017
SPECIES	P < 0.0005
INTERACTION	P < 0.01105

16.10.94 1 - 4 cm

TABLE 8: Determination of statistical significance for the final sampling at the 5-8cm level.

SOURCE OF VARIATION	STATISTICAL SIGNIFICANCE
FIRE HISTORY	0.25 < P
SPECIES	0.05 < P
INTERACTION	0.005 < P < 0.01

16.10.94 5 - 8 cm

TABLE 9: Determination of statistical significance for the final sampling at the 15-18cm level.

SOURCE OF VARIATION	STATISTICAL SIGNIFICANCE
FIRE HISTORY	0.25 < P
SPECIES	0.10 < P < 0.25
INTERACTION	0.025 < P < 0.05

16.10.94 15 - 18 cm

SITE		SIGNIFICANCE
DEPTH 1 - 4 cm		
16.10.94	0.0025 <P<0.005	SIGNIFICANT
9.10.94	0.005 <P< 0.01	SIGNIFICANT
5.10.94	P > 0.10	NOT SIGNIFICANT
DEPTH 5 - 8 cm		
16.10.94	0.05 < P < 0.10	NOT SIGNIFICANT
9.10.94	P > 0.10	NOT SIGNIFICANT
5.10.94	P > 0.10	NOT SIGNIFICANT
DEPTH 15-18 cm		
16.10.94	P > 0.10	NOT SIGNIFICANT
9.10.94	P > 0.10	NOT SIGNIFICANT
5.10.94	P > 0.10	NOT SIGNIFICANT

TABLE 9: SIGNIFICANCE BETWEEN *THEMEDA* UNBURNT AND *THEMEDA* BURNT

SITE		SIGNIFICANCE
DEPTH 1 - 4 cm		
16.10.94	P > 0.10	NOT SIGNIFICANT
9.10.94	P > 0.10	NOT SIGNIFICANT
5.10.94	P > 0.10	NOT SIGNIFICANT
DEPTH 5 - 8 cm		
16.10.94	0.05 < P < 0.10	NOT SIGNIFICANT
9.10.94	0.01 < P< 0.025	SIGNIFICANT
5.10.94	0.05 < P < 0.10	NOT SIGNIFICANT
DEPTH 15-18 cm		
16.10.94	0.01 < P< 0.025	SIGNIFICANT
9.10.94	P > 0.10	NOT SIGNIFICANT
5.10.94	P > 0.10	NOT SIGNIFICANT

TABLE 10: SIGNIFICANCE BETWEEN *NASSELLA* BURNT AND *NASSELLA* UNBURNT

SITE		SIGNIFICANCE
DEPTH 1 - 4 cm		
16.10.94	P > 0.10	NOT SIGNIFICANT
9.10.94	0.05 <P< 0.10	NOT SIGNIFICANT
5.10.94	P > 0.10	NOT SIGNIFICANT
DEPTH 5 - 8 cm		
16.10.94	P > 0.10	NOT SIGNIFICANT
9.10.94	P > 0.10	NOT SIGNIFICANT
5.10.94	P > 0.10	NOT SIGNIFICANT
DEPTH 15-18 cm		
16.10.94	P > 0.10	NOT SIGNIFICANT
9.10.94	0.005 <P< 0.10	SIGNIFICANT
5.10.94	P > 0.01	NOT SIGNIFICANT

TABLE 11: SIGNIFICANCE BETWEEN *NASSELLA* BURNT AND *THEMEDA* BURNT

SITE		SIGNIFICANCE
DEPTH 1 - 4 cm		
16.10.94	0.005 < P < 0.01	SIGNIFICANT
9.10.94	0.005 < P < 0.01	SIGNIFICANT
5.10.94	0.05 < P < 0.10	NOT SIGNIFICANT
DEPTH 5 - 8 cm		
16.10.94	0.005 < P < 0.01	SIGNIFICANT
9.10.94	0.005 < P < 0.01	SIGNIFICANT
5.10.94	0.05 < P < 0.10	NOT SIGNIFICANT
DEPTH 15-18 cm		
16.10.94	0.025 < P < 0.05	SIGNIFICANT
9.10.94	0.025 < P < 0.05	SIGNIFICANT
5.10.94	P > 0.10	NOT SIGNIFICANT

TABLE 12: SIGNIFICANCE BETWEEN *THEMEDA* UNBURNT AND *NASSELLA* UNBURNT

SITE		SIGNIFICANCE
DEPTH 1 - 4 cm 16.10.94 9.10.94 5.10.94	0.0025 <P<0.005 0.01 <P< 0.025	SIGNIFICANT SIGNIFICANT NOT SIGNIFICANT
DEPTH 5 - 8 cm 16.10.94 9.10.94 5.10.94	0.01 < P < 0.025 P > 0.10	SIGNIFICANT NOT SIGNIFICANT NOT SIGNIFICANT
DEPTH 15-18 cm 16.10.94 9.10.94 5.10.94	P > 0.10	NOT SIGNIFICANT NOT SIGNIFICANT NOT SIGNIFICANT

TABLE 13: SIGNIFICANCE BETWEEN *THEMEDA* UNBURNT AND ATRAZINE

SITE		SIGNIFICANCE
DEPTH 1 - 4 cm 16.10.94 9.10.94 5.10.94	0.005 <P<0.01 P> 0.10	SIGNIFICANT NOT SIGNIFICANT NOT SIGNIFICANT
DEPTH 5 - 8 cm 16.10.94 9.10.94 5.10.94	0.025 < P < 0.05 P > 0.10	SIGNIFICANT NOT SIGNIFICANT NOT SIGNIFICANT
DEPTH 15-18 cm 16.10.94 9.10.94 5.10.94	P > 0.10	NOT SIGNIFICANT NOT SIGNIFICANT NOT SIGNIFICANT

TABLE 14: SIGNIFICANCE BETWEEN *NASSELLA* UNBURNT AND ATRAZINE

SITE		SIGNIFICANCE
DEPTH 1 - 4 cm		
16.10.94	$0.0025 < P < 0.005$	SIGNIFICANT
9.10.94	$0.005 < P < 0.01$	SIGNIFICANT
5.10.94	$0.05 < P < 0.10$	NOT SIGNIFICANT
DEPTH 5 - 8 cm		
16.10.94	$P > 0.10$	NOT SIGNIFICANT
9.10.94	$P > 0.10$	NOT SIGNIFICANT
5.10.94	$P > 0.10$	NOT SIGNIFICANT
DEPTH 15-18 cm		
16.10.94	$P > 0.10$	NOT SIGNIFICANT
9.10.94	$P > 0.10$	NOT SIGNIFICANT
5.10.94	$P > 0.10$	NOT SIGNIFICANT

TABLE 15: SIGNIFICANCE BETWEEN *THEMEDA* UNBURNT AND *NASSELLA* BURNT

5. CONCLUSIONS

The results obtained here for the nutrient studies show no statistically significant difference in the soil nutrient (Total Kjeldahl Nitrogen and Total Phosphorus) concentrations of these two different grasses in these grasslands soils.

The results of the soil moisture analysis study indicated that mature stands of *Nassella trichotoma* effect the grassland soils by lowering the soil moisture content and out-competing the establishment of *Themeda triandra* from seed.

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APPENDIX 1

PREPARATION OF REAGENTS
- ANALYSIS OF PHOSPHORUS

POTASSIUM PERSULPHATE SOLUTION : Dissolve 50g Potassium Persulphate in 1000mL of distilled water.
If stored at room temperature in an amber bottle, the solution is stable for at least two weeks.

REAGENT A : 76.8g ammonium molybdate (A.R.) is dissolved in approx. 200 mL distilled water. 1.7554g antimony potassium tartrate is dissolved in approx. 100 mL distilled water. 896 mL 18 M sulfuric acid is dissolved in 500 mL of water. The first two reagents are added to the dillute sulfuric acid and the solution dilluted to 2 litres with distilled water. This solution is stored in a refrigerator.

REAGENT B : 1.7006g ascorbic acid is dissolved in 100 mL distilled water, 50 mL reagent A are added to 200 mL ith distilled water. This reagent must be prepared fresh daily.

STANDARD PHOSPHORUS SOLUTION : (50 ppm and 5 ppm) 0.2194g KH_2PO_4 is dissolved in approximately 100 mL distilled water, 5 mL 18M sulfuric acid added and the solution dilluted to 1 litre with distilled water. This stock solution contains 50 ppm P. 50 mL of this solution are dilluted to 500 mL to give a working standard of 5 ppm P.

APPENDIX 2

PREPARATION OF REAGENTS
- ANALYSIS OF NITROGEN

REACTION MIXTURE : 5g selenium, 5g copper sulphate ($Cuso_4$), 250g sodium sulphate (anhydrous) re mixed in a mortar and stored under dry conditions.

SODIUM HYDROXIDE : Dissolve 28g Sodium Hydroxide in 200 mL distilled water.

INDICATOR SOLUTION : Dissolve one vial of indicator mixture (0.1g) in 1000 mL of distilled water. Add 0.10 mL of 0.1 M NaOH.
The absorbance of this solution must be checked before use. The indicator solution must be prepared one day prior to use.