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EFFECT OF TEMPERATURE AND LITTER QUALITY ON DECOMPOSITION RATE OF *PINUS PATULA* NEEDLE LITTER



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A research report submitted to the Faculty of Science, University of the Witwatersrand, in partial fulfilment of the requirements for the degree of Master of Science

Johannesburg, March 2009

DECLARATION

I declare that this research report is my own, unaided work. It is being submitted in partial fulfillment for the degree of Master of Science in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other university.

_____ _____

Name of candidate Signature

Signed on _____ day of _____ 2009.

ABSTRACT

Decomposition of plant litter is an important component of the carbon and nitrogen cycles. Litter decomposition is regulated by the physical environment, litter quality and the nature and abundance of microbial communities. Climate has been found to exert strong controls over rates of litter decomposition and climate change may alter both carbon and nitrogen cycles. Global temperatures have increased by 0.74 ºC, and are predicted to increase by 1.1- 6.4 ºC within this century as a result of climate change, increases in temperature coupled with other climatic parameters are expected to affect litter decomposition rate.

This study examined the mass loss and CO_2 production from *Pinus patula* (Schlecht et Cham) leaf litter collected from fertilized plots in the Mpumalanga Province. The litter was incubated at various temperature regimes (15 ºC, 18 ºC, 24 ºC and 30 ºC) for 16 weeks. Litter decomposition increased with increasing temperature. Warming between 15 ºC and 18 ºC significantly increased the amount of CO_2 emissions from the litter; at 30 ºC there was a marked increase in the amount of CO_2 emitted. At the highest temperature there was a marked increase in the amount of CO_2 emitted from the litter; when compared to 15 ºC the amount of CO_2 evolved from litter incubated at 18 ºC was 58 % higher. Thus, future warming will increase the CO_2 emission from the forest floor. Mass loss of the litter was positively correlated with temperature levels with the highest temperature (30 ºC) recorded 41% more mass loss than 15 ºC. Nitrogen fertilizer applications had significant effects on litter decomposition rate but a minor effect on litter nitrogen quality. In addition, the residual effect of fertilizer was reflected in the nitrogen concentration of the litter and the decomposition rate. Nitrogen accumulation of the litter was positively correlated with temperature and nitrogen concentration in the original needle litter. The litter quality was not a strong predictor of litter decomposition rates implying that temperature is the major factor influencing the decomposition rate of *Pinus patula* needle litter. The results of this study are consistent with the hypothesis that the rate of nutrient cycling in non-limiting environments will increase, due primarily to an increase in litter decomposition as a result of increased temperatures.

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Chapter 1- Introduction

Litter decomposition forms an essential component of nutrient cycling processes and returns carbon (C) that was previously fixed in litter biomass as carbon dioxide (CO_2) into the atmosphere (Schimel, 1995, Wachendorf *et al.,* 1997). It is estimated that the nutrients released during litter decomposition can account for 67-87% of the total annual requirement of essential elements for forest plants (Waring and Schlesinger, 1985). Interest in the rate of litter decomposition has increased recently as it plays a major role in the dynamics of forest ecosystems.

The rate of litter decomposition is influenced by a number of factors including climate (temperature and moisture), litter quality and the nature and abundance of the decomposing organisms (Witkamp 1966a, Meentemeyer 1978, Swift *et al.,* 1979, Melillo *et al.,* 1982). According to Swift *et al.* (1979) and Berg *et al.* (2000) the two most important factors are climate and the chemical nature of the litter. Climate is the dominant factor in areas subjected to unfavourable conditions, whereas litter quality largely prevails as the regulator under favourable conditions (Couteaux *et al.,* 1995).

Different chemicals, their amounts and ratios have been shown to correlate with the decay rate of foliar litter and are used as substitutes for litter quality for example: nitrogen (N) (Hunt *et al.,* 1988, Heal *et al.,* 1997), Lignin (Meentemeyer 1978) and Lignin:N (Melillo *et al.,* 1982). However, there is no universal litter quality index because litter decomposition depends on qualities which differ among species (Taylor *et al.,* 1989) and plant parts. For example, Edmonds (1987) has reported higher decomposition rates for branches than twigs although their lignin concentration would not support this.

Geographical patterns of litter decomposition rates in major ecosystems types have been related to climate by Meentemeyer (1984). Results after 3 years of the Canadian Intersite Decomposition Experiment (CIDET), Moore *et al.* (1999) showed that lignin:N ratio, mean annual temperature and mean precipitation were valuable parameters for predicting mass loss but the data after six-years (Trofymow *et al.,* 2002) emphasized the dominance of climatic conditions over litter quality parameters in determining mass loss.

Temperature is often the primary factor determining rates of litter decomposition (Meentemeyer 1978, Hobbie 1996) and decomposition rates are generally more sensitive to temperature than are rates of primary production (Lloyd and Taylor 1994, Kirschbaum, 2000).

The global surface temperature is predicted to increase by 1.1-6.4 ºC within this century (IPCC, 2007) as a result of climate change and coupled with other climatic parameters is expected to affect the litter decomposition rate. Thus, under a warmer climate the balance between ecosystem C fixation and decomposition may be altered potentially causing a dramatic increase in the flux of CO_2 from the soil to the atmosphere (Schimel 1995, Cox *et al.*, 2000), even though the accuracy of any quantitative predictions of this flux is highly dependent on the assumed temperature sensitivity of decomposition (Couteaux *et al.*, 1995). Increases in temperatures are expected to increase decomposition rates, net N mineralization and nitrification (Peterjohn *et al.*, 1994, Hobbie 1996). Thus, elevated temperatures as a result of global warming have the potential to dramatically alter local ecosystems. In tropical regions, where high decomposition rates are compensated for by high primary production and lead to a relatively high soil C content understanding, the effects of increased temperatures on the C cycle are important especially in accessing the feedback of CO_2 to the atmosphere from these regions.

Currently most timber and pulp industries in South Africa rely heavily on managed plantations due to scarce natural forest resources, therefore investigating the effects of future temperature increases on decomposition rates of litter in plantation forest is important in understanding the nutrient dynamics and productivity of forests. Globally the trend of reliance on plantation forests is evident due to deforestation of natural forests and investigating the effects of increases in temperature on yield is of paramount importance. Another issue of importance globally concerning a rise in temperature is the possible increase in decomposition rate of litter which may significantly enhance global warming by increasing the release of CO_2 from litter or soil organic matter (Lloyd and Taylor 1994, Kirschbaum 1995, Liski *et al.*, 2003).

Rising atmospheric CO_2 concentrations can affect litter quality by altering tissue concentrations of nutrients (Norby *et al.*, 1986, Billes *et al.*, 1990) or refractory

compounds such as lignin (Norby *et al.,* 1986) and reduce decomposition rate. Other studies show increases in decomposition rate of litter grown under elevated CO_2 implying that the CO_2 effects on litter decay rates are sensitive to many factors, including initial litter quality (Cotrufo *et al.,* 1994, Cotrufo and Ineson 1995), plant nutrient availability (Kemp *et al.,* 1994) and soil fauna (Couteaux *et al.,* 1991, O'Neill 1994).

The forest floor is a key spot for coniferous forest functioning, with the largest natural above-ground inflow of organic matter and nutrients coming from the litter fall, dominated by tree foliage litter fall (Akselsson *et al.,* 2005) with global warming, elevated temperatures would have an impact on all biological processes and would be expected to increase decomposition rates and consequently, to reduce the forest floor mass (Yanai *et al.,* 2003 cited in Kurz-Bensson *et al.,* 2006).

There are few studies on substrate quality (chemistry) and decomposition of leaf litter of exotic trees species in South Africa or in the other parts of East or Southern Africa. One example is the work by Lisanework and Michelsen (1994) who studied decomposition and nutrient release in native forests and tree plantations using the litter bag technique. Another is a laboratory study by Lemma *et al.,* (2007) who studied substrate quality and decomposition of fresh leaf litter and fine roots of *Cupressus lusitanica, Pinus patula, Eucalyptus grandis* and native forest trees. In the Mpumalanga region of South Africa Dames (1996) studied biotic factors and altitude effects on litter accumulation and decomposition. As far as I know, no study has been done to evaluate sensitivity of litter decomposition to litter quality and temperature in the Mpumalanga region especially under anticipated climate change scenarios.

As most ecosystems exhibit some degree of nutrient limitation (Vitousek and Howarth, 1991) especially nitrogen (Shaver and Chapin 1986, Mc Nulty and Aber 1993) an understanding between fertilizer application (nutrient supply) and temperature response of decomposition is critical to long term predictions of ecosystem response to anticipated temperature increases as a result of climate change.

1.1 Objectives

The objectives of the present study were:

(1) To determine the initial concentration of nitrogen (N) and carbon (C) in needle litter of *Pinus patula* collected from compartments in which six different nitrogen fertilizer treatments were applied in 2001 and 2003.

(2) To characterize the changes in concentration of N and C of needle litter during the decomposition by comparing fresh litter with decaying litter.

(3) To determine the effect of temperature on decomposition rates of needle litter of *Pinus patula*.

(4) To determine the influence of litter quality on the decomposition rate of *Pinus patula* needle litter.

(5) To determine the effect of fertilizer application on the decomposition rate of *Pinus patula* needle litter.

Chapter 2- Literature review

2.1 Decomposition of litter

Decomposition of plant litter refers to the physical and chemical processes involved in reducing litter to its chemical constituents (Aerts, 1997). Decomposition of plant litter plays an important role in nutrient cycling and C fluxes of the terrestrial ecosystems (Swift *et al.,* 1979, Sun *et al.,* 2004). It also influences soil development and the availability of N, phosphorus (P) and other nutrients to plants and soil micro organisms (Huang *et al.,* 1998, Liu *et al.,* 2000).

Litter decomposition involves two simultaneous and fundamental set of processes: the concurrent mineralization and humification of lignin, cellulose and other compounds by a succession of micro organisms and the leaching downward in the soil of soluble compounds. Carbon and N are continuously mineralized or immobilized (Couteaux *et al.,* 1995). The decomposition processes progress in two stages after mass loss by leaching (Aerts, 1997, Chapin *et al.,* 2002). Labile and moderately labile compounds such as sugars, amino acids and cellulose are mainly decomposed during the early stage. In the latter stage, the recalcitrant compound of lignin is the dominant substrate for decomposition. During the later phase the decomposition process occurs slowly (Aerts, 1997, Berg, 2000) as lignin consists of very large and complex molecules which require more energy to decay.

In general decomposition of organic material involves initial immobilization (Knight *et al.,* 1994) of N and P followed by net nutrient release (Vitousek and Sanford, 1986) as the C to nutrient ratios change. McClaugherty *et al.* (1985) showed that during decomposition of hardwood leaf litter N concentrations increased linearly with cumulative mass loss which then eventually declined in some foliage litters. In *Pinus patula* plantations of the Mpumalanga lowveld it was found that N was rapidly released from decomposing material in the first 13 weeks of decomposition (Dames, 1996).

In terrestrial ecosystems, decomposition of litter is regulated by a host of factors including the litter's physical properties, climate and macro and micro fauna. According to Lavelle *et al.* (1993), these factors affect litter decomposition at different scales. Litter decomposition control factors operate in the following order: climate > litter quality > soil organisms. Meentemeyer (1978) and Swift *et al.* (1979) also consider climate and litter quality as the two most important factors controlling decomposition of the litter.

Effects of climate, such as temperature and precipitation, on litter decomposition rates are stronger in the early stage, while in late stages direct influence of climate on decomposition is reported to vanish and litter recalcitrance becomes more important (Johansson 1994, Edmonds and Thomas 1995, Berg 2000).

Decomposition rate varies among the species (Adams and Angradi 1996, Cornelissen 1996) and plant parts (Ostertag and Hobbie, 1999). Litter decomposition rate also varies with depth, for example a study by Moore *et al.* (2007) found pronounced decreases in decomposition rate from 10 to 60 cm depths: the k values fell from an overall average of 0.15 y^{-1} at 10 cm to 0.08 and 0.05 y^{-1} at 30 and 60 cm respectively.

The state of the forest also influences the rate of litter decomposition. Generally litter decomposition is faster in clear cuts than in undisturbed forests. Clear cutting often results in increased availability of nutrients (Smethurst and Nambiar 1990 cited in Prescott *et al.,* 2004). Additionally clearcuts have been associated with greater microbial activity resulting from warmer, moister conditions (Edmonds and McColl 1989). However, the influence of clearcutting on decomposition rates may differ with climate and depth of the forest floor (Binkley 1984).

Morphology of the litter also affects the decomposition rates. Broad leaf litter decomposes faster than needle litter which is attributed to higher nutrient concentration, lower lignin and polyphenol concentrations of broad leaf litter (Perry *et al.,* 1987). Cornelissen (1996) found that leaves of deciduous species decomposed twice as fast as those of evergreens under controlled conditions. However, other studies have not consistently reported faster decomposition or N mineralization of broad leaf litter compared with needle litter (McClaugherty *et al.,* 1985).

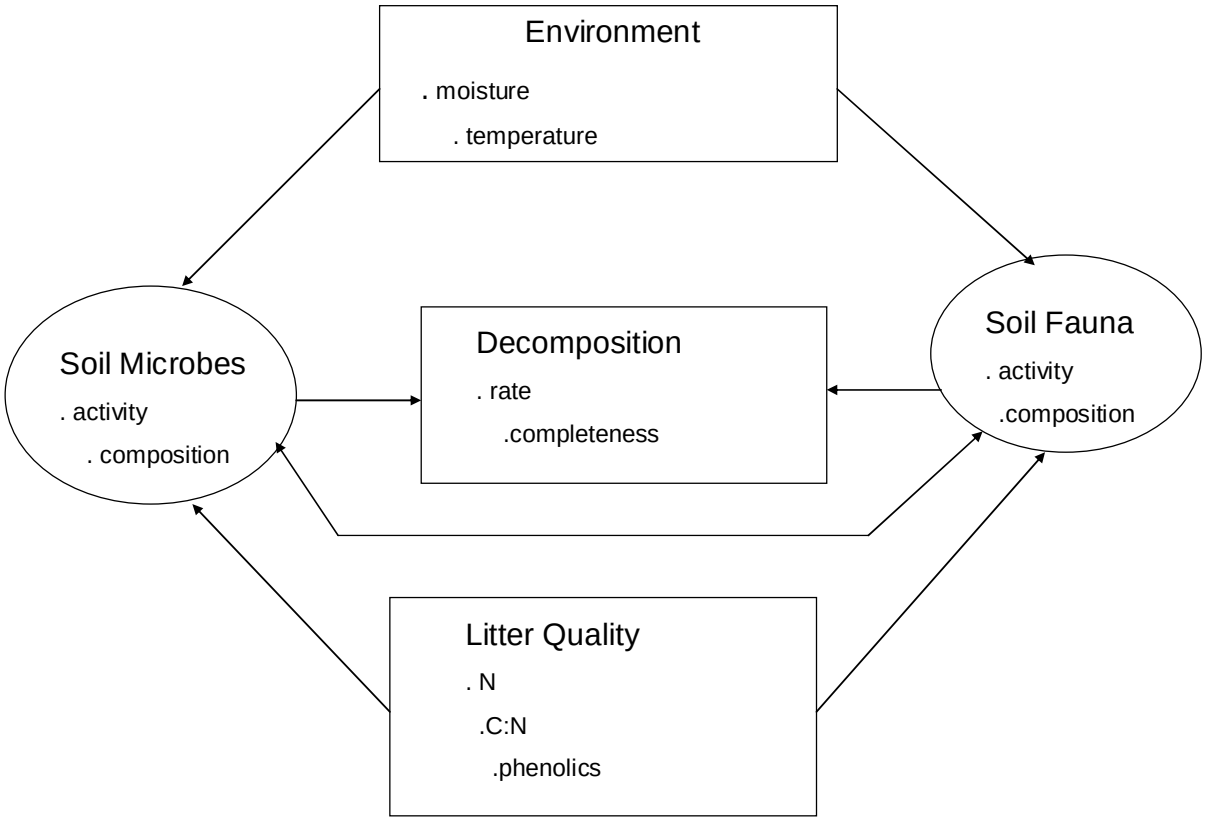


Figure 2.1 Factors that control the rate and completeness of decomposition. Source: Prescott *et al.* (2000).

2.2 Elevated Temperature

The global surface temperature has increased in the past 100 years by about 0.74 ºC and is recently predicted to increase by 1.1-6.4 ºC within this century (IPCC, 2007) and as surface temperature increases soil temperature can be expected to rise concomitantly (Schlesinger and Andrews, 2000). The temperature increase is expected to directly alter ecosystem process such as C and N cycling (Bonan and Van cleve 1992, Jonasson *et al.*, 1993) and may modify litter quality, litter decomposition rates and nutrient mineralization rates that control the availability and cycling of nutrients (Hobbie, 1996).

Increased temperature may also alter plant species composition (Chapin and Shaver 1985, Wookey *et al.,* 1993, Chapin *et al.,* 1995) and hence affect litter production and quality indirectly through species-specific responses (Cornelissen, 1996). Increasing temperatures could increase rate of litter decomposition directly by increasing microbial activity, releasing C and N from the soil organic matter (Shaw and Harte, 2001). Analysis of the control of decomposition across ecosystem types shows that much of the variation is explained by temperature and moisture (Ryan *et al.,* 1990, Berg *et al.,* 1993). With increased temperature, litter moisture could decline and reduce decomposition rate (Berg *et al.,* 1993, Robinson *et al.,* 1995), even though the degree to which temperature and moisture control decomposition across ecosystem types will depend on the litter quality, the degree of climatic variability and severity (Berg *et al.,* 1993).

Global warming, in response to rising concentrations of atmospheric gases that absorb infrared radiation, and associated environmental changes are predicted to mostly affect arctic regions and will be particularly pronounced at high northern latitudes this century (Maxwell, 1992, and ACIA, 2005 cited in Cornelissen *et al.,* 2007). Potential consequences of such warming for C and nutrient-cycling in arctic soils have received considerable attention for several reasons. According to Billings (1987) large stocks of C are located in boreal and arctic regions and warmer temperatures could potentially stimulate mineralization of this C, creating a positive feedback to rising concentrations of atmospheric CO_2. Additionally, if increased decomposition of soil organic matter results in greater nutrient availability for plants, net primary production (NPP) could increase with warming, since NPP is strongly nutrient limited in the tundra region (Shaver and Chapin, 1986 cited in Hobbie, 1996). The changes in NPP associated with both warmer and wetter climates and elevated atmospheric CO_2 concentrations are probably of greater significance to the C budget of forests.

Melillo *et al.* (1993) used a process based terrestrial ecosystem model to predict global changes in NPP for major vegetation types. They showed that changes in climate and CO_2 may lead to an increase in NPP of 30-40% in boreal forests and woodlands, whereas an increase was predicted to be between 5 and 15% in temperate forests. For tropical

evergreen forests a decrease of between 4.5-12.9 % is predicted due primarily to P limitation. As increased NPP leads to an increased rate of litter fall, this will increase the store of C both in vegetation and on the forest floor, especially if the net increase in decomposition rate is minimal.

Changes in atmospheric composition in the tropics are essentially the same as those in higher latitudes, despite differences in the source and sink strengths for the trace gases. In the tropics radiant energy is abundant but soils are frequently nutrient-deficient rather than C constrained, therefore the process limiting the rate of carbon cycling through the ecosystem is decomposition (Scholes and Breemen, 1997).
Because of difficulties and large uncertainties in estimating the temperature sensitivities of decomposition of soil organic matter pools, the relationship between the temperature sensitivity of decomposition and soil organic matter pools is of paramount interest (Reichstein *et al.,* 2005) as it is the central link between climate change and the global carbon cycle especially feedback of CO_2 into the atmosphere (Kirschbaum, 2006).The Climate-Carbon Cycle model (Cox *et al.,* 2000) showed a strong positive feedback, which turned the land biosphere from a sink to a source around the year 2050. Other models resulted in a positive but much weaker climate-carbon cycle feedback (Friedlingstein *et al.,* 2006). According to Raddatz *et al.* (2007) the tropical land biosphere is expected to dominate positive feedback of CO_2 to the atmosphere due to a reduction in NPP. Not only do deeper and older C pools contribute importantly to such feedbacks, but so do recently formed labile C pools, particularly litter (Grogan *et al.,* 2001 cited in Cornelissen *et al.,* 2007).

Decomposition of litter (including root litter) is noted for contributing about 70% of the total annual C flux (Raich and Schlesinger, 1992 cited in Aerts, 1997) and mineralization of the annual litter fall according to Couteaux *et al.* (1995) contributes to approximately half of the CO_2 output from the soil and notes that this proportion remains stable because of its relatively constant annual input.
Sensitivity of soil organic matter and litter to increasing temperature has received much attention especially in assessing whether the increase in temperature will result in

increase in mineralization of essential elements like C and N and feedback of CO_2 into the atmosphere.

In a number of studies conducted in temperate, deciduous and boreal forests there was a positive correlation between increased N mineralization rates, CO_2 evolution and increased temperature (Witkamp, 1966a; Hobbie, 1996; Osono and Takeda, 2006). However, in some in sub-arctic areas much of the mass loss of the litter occurs in the winter months under snow cover where the temperature ranges from -5 ºC to +1 ºC (Moore, 1984).

The effect of increased temperature on decomposition rate has been found to vary with the duration of exposure. For example with prolonged warming, warming effects on soil organic matter decomposition have been shown to decline with time (Luo *et al.,* 2001). This decline was previously explained as the increase in the proportion of the resistant pool at later stages with the resistant pool being less sensitive to warming (Peterjohn *et al.,* 1994), or as an adaptation of the microbial community to enhanced temperature (Luo *et al.,* 2001).

The forest floor is a key spot for coniferous forest functioning, with the largest natural above-ground inflow of organic matter and nutrients coming from the litter fall, dominated by tree foliage litter fall (Akselsson *et al.,* 2005). Under *Pinus patula* plantations at high altitudes, plant litter, such as needles and branch material can accumulate and reach levels up to 216.7 tons ha^{-1} (Schutz *et al.,* 1983). In the central Ethiopian highlands, a mean litter thickness of 14 cm was observed in *Pinus patula* stands (Yirdaw and Luukkanen, 2003) and an average litter thickness of 10 cm (range 3-35 cm) was observed in a study in the republic of South Africa in *Pinus patula* plantation (Schutz, 1990 cited in Dames *et al.,* 1998) with global warming, a rise in temperature would be expected to increase decomposition rate and reduce the forest floor mass (Yanai *et al.,* 2003 cited in Kurz-Bensson *et al.,* 2006).

2.3 Litter quality

Litter quality refers to the chemical components of the material and has been defined in various ways: litter nutrient concentration (Taylor *et al.,* 1989, Stump and Binkley 1993, Berg *et al.,* 1996), soluble C fractions (Hobbie, 1996), insoluble C fractions such as lignin (Meentemeyer, 1978), the ratio of lignin to nutrient (Melillo *et al.,* 1982, Hobbie, 1996) and the concentration of phenolics (Aerts and De Caluwe 1997, Steltzer and Bowman, 1998).

In many studies, N content (Hunt *et al.,* 1988, Aber *et al.,* 1990), or more usually the C:N ratio (Taylor *et al.,* 1989, Aerts, 1997) has been shown to be a valuable predictive tool of litter decomposition. Theoretically, the optimum C:N ratio for microbial growth is approximately 25 (Swift *et al.,* 1979), but fungi and bacteria can decompose substrate with much higher ratios (Heal *et al.,* 1997). Substrates with a C:N ratio of <20 decomposes rapidly and ones with an intermediate C:N ratio of 25-75 can also decompose quickly, but N mineralization activity is often reduced by increased microbial immobilization as well as protein complexation by polyphenols (Silver and Miya, 2001). Substrates with high C:N ratios (>75) are often much more difficult to break down and are generally characterized by greater amounts of structural woody materials such as condensed tannins and terpenes, as well as low available N for decomposer organisms (Heal *et al.,* 1997). Several other features of undecomposed leaf litter are negatively associated with the decomposition rate. These features include lignin content (Meentemeyer, 1978, Taylor *et al.,* 1991) and lignin:N ratios (Melillo *et al.,* 1982, Aerts, 1997, Ostertag and Hobbie, 1999). Other studies have found limitation of decay rate by P content or the C:P ratio (Schlesinger and Hasey, 1981, Staaf and Berg, 1982).

Decomposition rate of leaf litter depends greatly on the physico-chemical properties of leaves which have different degree of influence. Nitrogen for example is one of the commonest factors limiting litter decomposition as it determines the growth and turnover of the microbial biomass mineralizing the organic C (Heal *et al.,* 1997).

Van Cleve's 1974 review of decomposition studies from circum polar tundra and taiga sites (cited in Meentemeyer, 1978), reports that P + Ca concentration, lignin + tannin and carbohydrates including cellulose all have significant correlations with decomposition

rates. He suggests that the dominant influence which lignin apparently exerts over decomposition rates may result from its ability to serve as a surrogate for the many physical and chemical properties which regulate their decomposition rate.

Lignin and N content of the litter has been found to control decomposition rate at different stages. Nitrogen content is an important rate-regulating factor in the first stages of decomposition (Cotrufo *et al.,* 1995), while in later stages lignin concentration becomes a better predictor of decomposition rates (Berg, 1984).
Litter quality characteristics are most useful in describing litter decay rates in environments where climatic and edaphic variables are held relatively constant (Swift *et al.,* 1979). Litter quality variables exert little or secondary control on litter decay rates in ecosystems where this is not the case, particularly in extreme environments (Schaefer *et al.,* 1985). In ecosystems where temperature and moisture availability control ecosystem functions, including microbial activity they will exert strong controls over litter decomposition and decay rates (Swift *et al.,* 1979). For example, patterns of litter decomposition at very dry sites have been found to be influenced more by moisture variation than by litter quality (De Santo *et al.,* 1993, Murphy *et al.,* 1998).

The relative control of lignin over decomposition rate is not uniform over different climatic regions. According to the Couteaux *et al.* (1995) study, in late decomposition stages, the effect of higher lignin concentrations was low in harsh climates for example at the arctic circle, whereas when the same substrate was decomposed in warmer and wetter regions like an European-Atlantic climate, higher lignin concentrations had a predominant effect. In a wet tropical climate where temperature and moisture are less constraining, litter decomposition rate was found to depend primarily on soil and humus properties and litter quality (Bargali *et al.,* 1993). Meentemeyer (1978) generally found that the greater the abundance of energy and moisture as indicated by Actual-Evapotranspiration the faster the decay rate for a given lignin content and the higher the lignin content the more the energy and moisture is required to cause breakdown.

Predicting litter decay rate using either lignin: N ratio and C:N ratio has been a topic of debate. For example a study by Hobbie (1996) found that decomposition of litter in tussock tundra is controlled by litter C quality (lignin and carbohydrates) concentration rather than litter N concentration. Other studies (Melillo *et al.*, 1982, Aerts, 1997 and Meentemeyer, 1978) also favour lignin content as the best predictor of decay rate. Voigt (1965), Daubenmire and Prusso (1963) laboratory studies of hardwood and conifer leaves incubated at 32 ºC and 10 ºC respectively found a positive correlation between weight loss and initial N content, even though the correlations were weak, r=0.3691, 0.4782. Taylor *et al.* (1989) using leaf litter found only limited support for the lignin:N ratio as an index of substrate quality. Under most circumstances their findings revealed C:N ratio or N content alone provides better predictions of mass loss rates than did the lignin:N ratio. They further claim that lignin control of decay rates may be absent or weak in a litter of certain species due to relatively low lignin concentration in that litter. However, several studies have not found significant correlation between decomposition rates and chemical litter quality parameters: C:N, C:P, lignin concentration and lignin:N (Moore 1984, McClaugherty *et al.*, 1985, Moore *et al.*, 2007, Castanho and Oliveira, 2008).

2.4 Microorganisms

Microorganisms also control the rate of litter decomposition and nutrient dynamics (Seastedt 1984, Herughan and Bolger 1998). Nutrients in the litter are mineralized through the action of decomposer organisms and become available for uptake by the plant community (Swift *et al.*, 1979).

Decomposition of plant material is largely mediated by fungi and bacteria, which usually have lower C:N ratios and C:P ratios than the litter on which they grow (Cotrufo *et al.*, 1999). These microorganisms therefore have high requirements for these nutrients, and detritus with high concentrations of N and P will decompose faster than detritus with low N and P concentrations, because of the associated faster growth of decomposer populations (Enriquez *et al.*, 1993) as low C:N and C:P values are often associated with

nutritious and succulent leaves, whereas high values often coincide with tough leaves which are high in resistant components such as cellulose and lignin (Witkamp, 1966b).

Generally the early colonizers of coniferous leaf litter are bacteria, ascomycetes, deuteromycetes and some basidiomycetes, which attack simple carbohydrates and cellulose. These are followed by phycomycetes, particularly members of the mucorales which can utilize the fungal breakdown products, in combination with the meiofauna (Millar, 1974). Fungi are reported to perform a dominant role in litter decomposition because they decompose the lignocellulose matrix in litter that other organisms are unable to decompose (Kjøller and Struwe, 1982). Study by Read *et al*. (2004) found that some species of ericoid and ectomycorrhizal fungi can produce extracellular enzymes that decompose cellulose, hemicellulose, starch as well as recalcitrant compounds such as polyphenols. Ectomycorrhizas fungi has also been found to utilize a range of amino acids and protein as their sole source of N and providing host plant access to organic N (Dames *et al*., 1999). Andersson *et al*. (1997) has also demonstrated uptake of N from a protein source by mycorrhizal plants of *Betula pendula, Picea mariana, Pinus contorta, Pinus sylvestris, Eucalyptus grandis* and *Eucalyptus maculata*. In the Mpumalanga province of South Africa different species of ectomycorrhizas have been found to occur at different stand age of the trees. *Amanita spp*. and *Lycoperdon spp*. occur under younger trees (up to 15 years) and *Boletus spp*. and *Suillus spp*. occur in older stands while *Scleroderma spp*. occur mainly under stand from 10 years onward (Dames *et al*., 1999).

Microbial populations during litter decomposition depend on various factors. When a sample of freshly fallen litter is incubated in the laboratory under optimum moisture and temperature conditions, the bacterial numbers usually show strong increases during the initial stages of the experiment, correlated with an increase in pH and disappearance of soluble organic components. Often a peak in numbers is reached within a few weeks, and then the numbers gradually decrease again. Litter with an initially high pH shows the most rapid increase and reaches the highest maximum numbers (Witkamp, 1963). In a study by Witkamp (1966b), which involved counting of both bacteria and fungi in litter from several different tree species in North America, decreases were found of both bacterial and fungal counts with an increase in the C:N ratio of the litter.

Prevailing environmental factors influence the microbial activity of which moisture and temperature are the two main factors noted for affecting microbial activity (Singh and Gupta, 1977). The litter decomposition rates may change owing to direct effects of climate change on microbial activity and/or to indirect effects on microbial activity through the changing of litter quality (Hobbie 1996, Cornelissen *et al.,* 2007). Microbial activity is also affected by activity of larger animals on the litter. With the lack of mechanical breakdown by larger animals there will be less surface available for microbiological attack and hence decomposition rate will be retarded (Cortez and Bouche, 1998).

2.5 Elevated CO_2

There is ongoing debate about whether the increasing atmospheric concentration of CO_2 is resulting in increases in net terrestrial ecosystem carbon (C) storage (Melillo *et al.,* 1996 cited in Norby *et al.,* 2001). Numerous studies have shown conflicting evidence for retarded decomposition and N mineralization rates in elevated CO_2 grown litter. Several studies have found that decomposition rates of tissues grown at elevated CO_2 concentrations are slower than those grown under ambient concentration: in *Lolium* roots (Gorissen *et al.,* 1995), in marsh Scirpus (Ball and Drake, 1997) and *Betula pendula* leaves (Cotrufo and Ineson, 1996), Boerner and Rebbeck (1995) report a decrease in both mass loss and N release in yellow poplar leaves from seedlings grown in elevated CO_2. Cotrufo *et al*.1995 report a decrease in mass loss of elevated CO_2 birch roots but no change in elevated CO_2 spruce roots.

Reduction in decomposition rate is attributed to change in the lignin: N matrix (Norby *et al.,* 1986) and changes in allocation of N to different tissues (Norby *et al.,* 1986 Billes *et al.,* 1990). Cotrufo *et al.* (1998) study under elevated CO_2 showed that plants changed their allocation of N between above and below-ground components: root N concentrations were reduced by an average of 9% compared to 14% average reduction for above ground tissues. Elevated atmospheric CO_2 increases the ratio of root litter to shoot litter (Cotrufo and Ineson 1996), increases the rate of microbial immobilization

(Bernston and Bazzaz 1998) and alters soil microfaunal interactions (Melillo *et al.*, 1982, Couteaux *et al.,* 1991).

Other studies found no significant effect of elevated CO_2 on decomposition rate. Hirschel *et al.* (1997) for example found little difference in lignin:N ratio of senesced leaf litter grown under elevated CO_2 concentrations, and there was little difference in decomposition rates, compared with materials grown under ambient CO_2 concentrations. Hall *et al.* (2006) found that concentrations of carbon, hemicellulose and lignin were higher in litter derived from material grown under elevated CO_2 but they found that litter from ambient and elevated CO_2 decomposed at comparable rates. However, the atmosphere in which the decomposition took place resulted in significant differences in rates of decomposition. Litter decomposing under elevated CO_2 decomposed more rapidly than litter under ambient CO_2 and exhibited higher rates of mineral N accumulation. Kemp *et al.* (1994) found no significant effects of elevated CO_2 on decomposition of *Andropogon gerardii*, *Sorghastum nutans* and *Poa pratensis* litter produced in tall grass prairie. Couteaux *et al.* (1991) report a retarded weight loss of elevated CO_2 sweet chestnut leaf litter decomposed in soil of low fauna diversity but an increased weight loss with more complex soil fauna. Robinson *et al.* (1997) found that decomposition rates of *Festuca* shoots and leaves grown at elevated CO_2 were slower than ambient CO_2 samples when placed in a high arctic site, but the difference was reversed or insignificant at a low Arctic site. Parsons *et al.* (2004) found that mass loss rates were higher for litter from ambient CO_2 than that of elevated CO_2. Studies that investigated the effect of elevated CO_2 on litter quality reported contrasting results. Meta-analyses of data from naturally senesced leaves in field experiments by Norby *et al.* (2001) showed that the N concentration in leaf litter was 7.1% lower in elevated CO_2 compared to that in ambient CO_2 and in Oak, leaf lignin concentration increased with an increase in CO_2 (Cotrufo *et al.,* 1999). Other studies also noted reduction in foliar N concentration with elevated CO_2 (Norby *et al.,* 1986, Couteaux *et al.,* 1996, Hall *et al*; 2005). In other studies litter quality remained unchanged depending on the system (Franck *et al.,* 1997, Norby *et al.,* 1999). Henry *et al*. (2005) found that lignin concentrations increased in grass and forb litter under elevated CO_2, however they found

no differences in total phenolic concentrations or percent nitrogen of the litter grown under elevated CO_2 environment. Nutrient supply also affects the response of the biosphere to elevated CO_2. Elevated CO_2 caused an increase in litter C:N ratio in *Lolium* shoots and roots and a decreased shoot C:N in *Avena* at high nutrient status (Franck *et al.,* 1997).

The effect of elevated CO_2 on soil and litter microorganism abundance shows no general trend. Elevated CO_2 decreased the abundance of microarthropods under deciduous trees (Loranger *et al.,* 2004) and the abundance of microarthropods and nematodes in loblolly pine plantations (Hansen *et al.,* 2001, Neher *et al.,* 2004). On the other hand Markkola *et al.* (1996) found no consistent responses of soil fauna to doubled atmospheric CO_2 concentration in a pot experiment with Scot pines and coniferous forest humus. However, Carney *et al*. (2007) found that soils exposed to elevated CO_2 had higher relative abundance of fungi and higher activities of a soil C degrading enzyme, which led to more rapid rates of soil organic matter degradation than soils exposed to ambient CO_2.

The effects of elevated CO_2 on litter decomposition studies showed no uniform response indicating that CO_2 effects on litter decay rates are influenced by many factors including initial litter quality (Cotrufo *et al.,* 1994, Cotrufo and Ineson, 1996), plant nutrient availability (Kemp *et al.,* 1994, Franck *et al.,* 1997), plant species (Bazzaz *et al.,* 1990, Franck *et al.,* 1997) and soil fauna (Couteaux *et al.,* 1991, O'Neill 1994).

C:N ratio of the litter was found to be a good predictor of mass loss and nutrient release for the litter produced under elevated CO_2 (Taylor *et al.,* 1989, Cotrufo *et al.,* 1994). Cotrufo *et al*. 1995 also reported that the C:N ratio was a better litter quality index in explaining mass loss of birch and spruce roots than % N.

2.6 Time effect

Decomposition of the litter is affected by time together with other factors including litter quality, physical environment and microorganisms. The loss in dry mass during decay has been estimated by various studies under different conditions and different time spans. Hayes (1965), working under laboratory conditions with *Picea sitchensis* and *Abies*

grandis, noted decreases of 20-30% in the first two months and 50% in the 16 months of incubation. Loss in dry weight was 24% after 2 years and 47% after 7 years in the stand of *Pinus sylvestris* examined by Kendrick (1959).

Li *et al.,* (2006) using *Dacryodes excelsa* leaf litter found that mass loss rate declined with the incubation time. Leaf litter decayed fast during the first 3 months and after 8 months of incubation the leaf mass loss slowed down and approached a constant value through the next 4 months of the study period. Data for four types of litter from a blanket bog in northern England showed a significant decline in the rate of respiration with increasing decomposition in the leaf litter which had high initial rates of respiration and large soluble sugar contents (*Rubus chamaemorus* and *Eriophorum vaginatum*). The calluna shoots and stems showed no significant decline in respiration rate in relation to stages of decomposition even though these were litters with slow rates of respiration and weight loss (Heal *et al.,* 1978 cited in Swift *et al*., 1979).

Witkamp (1966a) found a slight increase in the rate of respiration with time in a seasonal study of four species of tree litter but he suggested that this resulted from counteracting the influence of chemical impoverishment and improving physical conditions.

Resource quality of the litter has been found to change with time of decomposition. Van der Linden (1971) cited in Swift *et al*. (1979) showed that over a two-year period of decomposition of hazel leaves, while the total immobilized N increased there was a decrease in the amount that could be mineralized by a proteolytic enzyme from *Streptomyces griseus*.

Another feature of the progress of decay is the formation and accumulation of humic materials which normally has a slow rate of decomposition (Swift *et al.,* 1979).

2.7 Fertilizer effect

Fertilization can directly affect forest floor nutrient pools by either increasing nutrient content of the soil or that of the litter, thus influencing the decay rate and nutrient release dynamics (Tietema 1993, Aerts and De Caluwe 1997, Sanchez, 2001).

Studies concerning the effects of fertilization (N addition) on rate of litter decomposition, litter nutrient concentration and nutrient release dynamics have been controversial. Some

studies (Hunt *et al.*, 1988, Ostertag and Hobbie 1999, Li *et al.*, 2006) found that leaf litter from fertilized plots decomposed faster than that from the control plots, others found no effect (Staaf 1980, van Vuuren and van der Eerden 1992, Prescott 1995, Dukes and Field 2000, Gurlevik *et al*. 2003), while others reported depressing effects of fertilization on decomposition rate of the leaf litter (Titus and Malcolm 1987, Kemp *et al.*, 1994, Magill and Aber 1998). Fertilization has also been found to affect decomposition rate differently at different decomposition stages. Berg *et al.* (1982) for example found a positive response to N in the initial decomposition phase but a negative response in the later stages. The type of fertilizer application has been found to affect differentially decomposition of soil organic carbon. For example study by Bradford *et al*. (2008) found that P amendment stimulated decomposition rates while N fertilization suppressed decomposition of organic matter.

Leaf litter nutrient concentration in some studies have been found to be higher in fertilizer stands than the control (Robinson *et al.*, 1995, Finer 1996, Vitousek 1998) although opposite results have also been reported (Pastor *et al.*, 1987, Aerts and De Caluwe 1997). Increased N supply may also affect the concentrations of lignin and phenolics of the litter. However, these effects appear to be species-specific (Harborne 1997, Aerts and De Caluwe 1997).

Chapter 3- MATERIALS AND METHODS

3.1 Study area

Litter was collected from 18-year old *Pinus patula* (Schlecht et Cham) plantations located in the Ngodwana SAPPI plantation forest in the Mpumalanga province of South Africa (25^0 34' S, 30^0 38' E). The province is situated in the eastern part of South Africa; it is a summer rainfall region with precipitation occurring mainly in the form of thunderstorms. The mean annual rainfall varies from 350 mm in the north east to 1600 mm on the escarpment. The region's proximity to the tropic of Capricorn and warm Mozambique current of the Indian Ocean results in a subtropical, frost-free climate in the low lying areas of the lowveld (Schulze, 1972).

3.2 Study sites

The study plots were located in Mamre, Elandshoogte and Mooifontein plantations. The criteria for selection were based on the fact that fertilizer trials were taking place in these plantations and fertilization was anticipated to affect the litter quality. Details of the study sites with respect to plantation, compartment, land type, site quality, dominant geology, altitude, soil texture class, mean annual temperature (MAT) and mean annual precipitation (MAP) are given in Table 3.1. Site quality and land type are indices that have been developed by the forestry industry to indicate potential productivity. The value of 3 depicts sites with better productivity than sites with values of 4. Incase of land type value 400 depict better productivity than 600.



Figure 3.1 Map of the study sites

Table 3.1 Details of the study sites

Trial Number	Plantation	Compartment	Geology	Soil texture	Site quality	Altitude(m)	MAP (mm/yr)	MAT (^{o}C)	Land type
LM027	Mamre	K37	Shale	CILM	4	1600-1800	1117	14.7	600
LM028	Elandshoogte	A125	Andesite	CI	3	1600-1800	897	14.6	600
LM029	Mooifontein	B36	Mixed shale/arenite	CILM	4	1400-1600	1174	15.7	400

Where CI= Clay soil and CILM= Clay loamy soil. (Pallet, 1991).

3.3 Experimental design

Needles were collected from second rotation *P. patula* plots planted in 1989. The study plots underwent six fertilizer treatments and two replicates were used for each treatment. Only two replicates could be accommodated in the field design. The treatments were:

(1) Control: no fertilizer additions (T1);

(2) 100kg/ha Limestone ammonium nitrate (LAN) 28 % (N) at 11 years old (T2);

(3) 100kg/ha Urea 46 % (N) at 11 years old (T3);

(4) 100kg/ha LAN 28 % (N) at 13 years old (T4);

(5) 100kg/ha LAN 28 % (N) at 11 and 13 years old (Total of 200kg/ha) (T5) and

(6) 100kg/ha Urea 46 % (N) at 11 and 13 years (Total of 200kg/ha N) (T6).

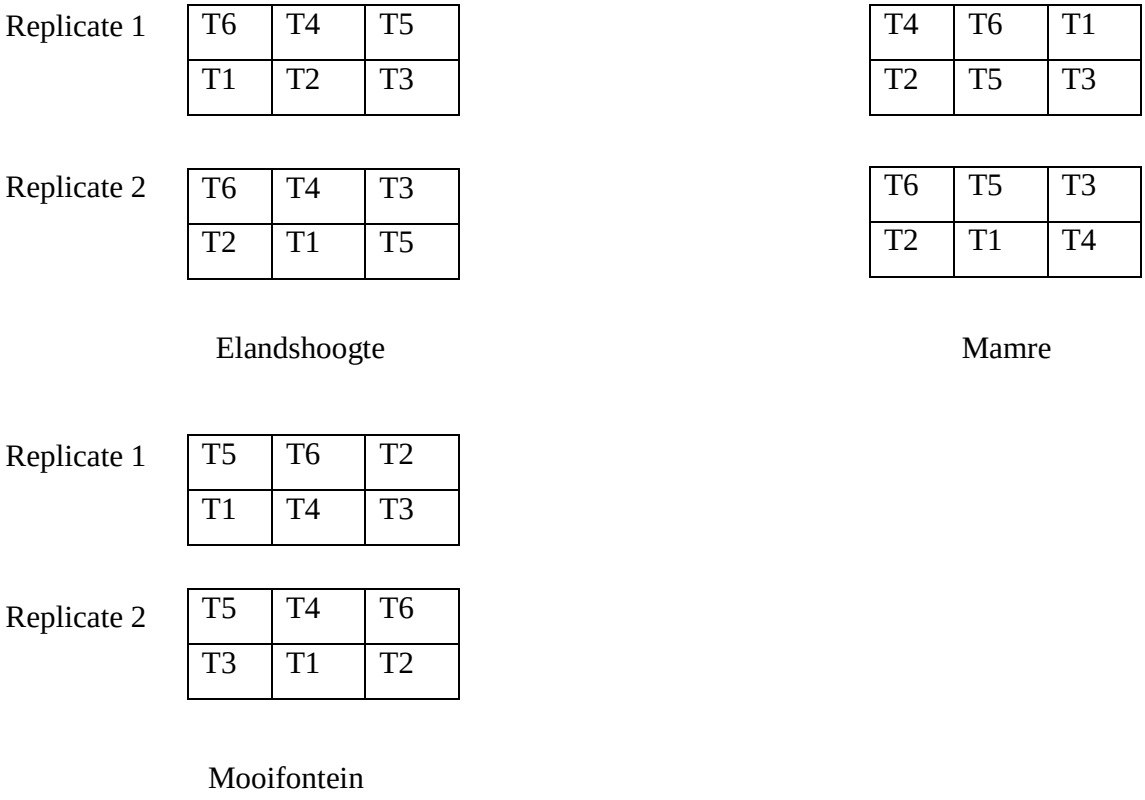


Figure 3.2 Plot layout

The main period of litter fall in this area is between March and September (Dames *et al.*, 1998) reaching a peak at the time of sampling (September).

3.4 Litter collection

Newly shed needles were collected from *P. patula* plots in September 2007 which were subjected to six fertilizer treatments. The size of each plot is 576 m^2, containing 11 × 11 trees with a spacing of 2.4 × 2.4 m. A bulked sample of the surface litter, made up of five quadrats (each of size 0.25 m by 0.25 m) randomly placed were collected from each site, that is 36 samples (6 treatments × 2 replicates × 3 sites) in total. The litter was packed in paper bags and transported to the laboratory.

3.5 Needle litter decomposition

The *P. patula* needles were weighed into 10 g samples and each sample inserted into 300 ml glass jars. The glass jars were covered tightly with screw caps and the needles were incubated at four temperature regimes (15 º, 18 º, 24 º and 30 ºC) for 16 weeks. These temperatures were chosen to reflect current ambient (15 ºC), a likely change with global change (18 ºC) and extreme temperatures to measure the upper limits of decomposition rates. Subsamples of fresh needles collected from each plot were oven dried to constant mass at 65 ºC for 72 h and oven dry/fresh mass ratio was used to determine initial moisture content to convert fresh needle mass to dry weight and to express the decomposition parameters on corrected on a dry mass basis.

After 2, 5, 6, 8 and 16 weeks of incubation, two jars (representing two replicates) per each fertilization regime for three sites at four temperatures were retrieved (144 jars at each sampling date) and the needles were oven dried. Litter mass loss was determined by weighing oven dried samples and subtracting their mass from corrected oven dried mass.

After 2, 6, 8, and 16 weeks the rate of CO_2 production was measured. To measure CO_2 flux from the needle litter at each incubation period, jars were removed (144 jars per each sampling date), opened and aerated for 10 minutes to release the CO_2 evolved. This approach was used because during a pilot experiment 0.1 M KOH solution used to capture CO_2 became saturated after 1 or 2 weeks of incubation hence a 2 hourly reading used at the end of each incubation period. The reasoning was that using this approach an

instantaneous measure of CO_2 emission could be calculated, thus CO_2 data presented are not cumulative over the incubation period. The assumption is that the data are comparative across treatments, temperatures, time and sites using this approach. Test tubes containing 18 ml of 0.1 M KOH were inserted into the jars and closed tightly with screw caps and returned to the incubators. Controls were also set up by inserting test tubes into jars without needles. After 2 hours, test tubes were removed and the content titrated with 0.1 M HCl after addition of saturated $BaCl_2$ solution (1.44 ml per 18 ml KOH) using phenolphthalein as an indicator. The difference in HCl consumption between the blank and the sample indicated the amount of adsorbed CO_2. Carbon dioxide emitted was calculated on the basis that 1 ml 0.1 M HCl is equivalent to 2.2 mg CO_2 (Anderson and Ingram, 1993). Thus, the experimental design consisted of a factorial combination of three sites, six treatments, two replicates, four temperature regimes and five sampling dates, a total of 720 jars.

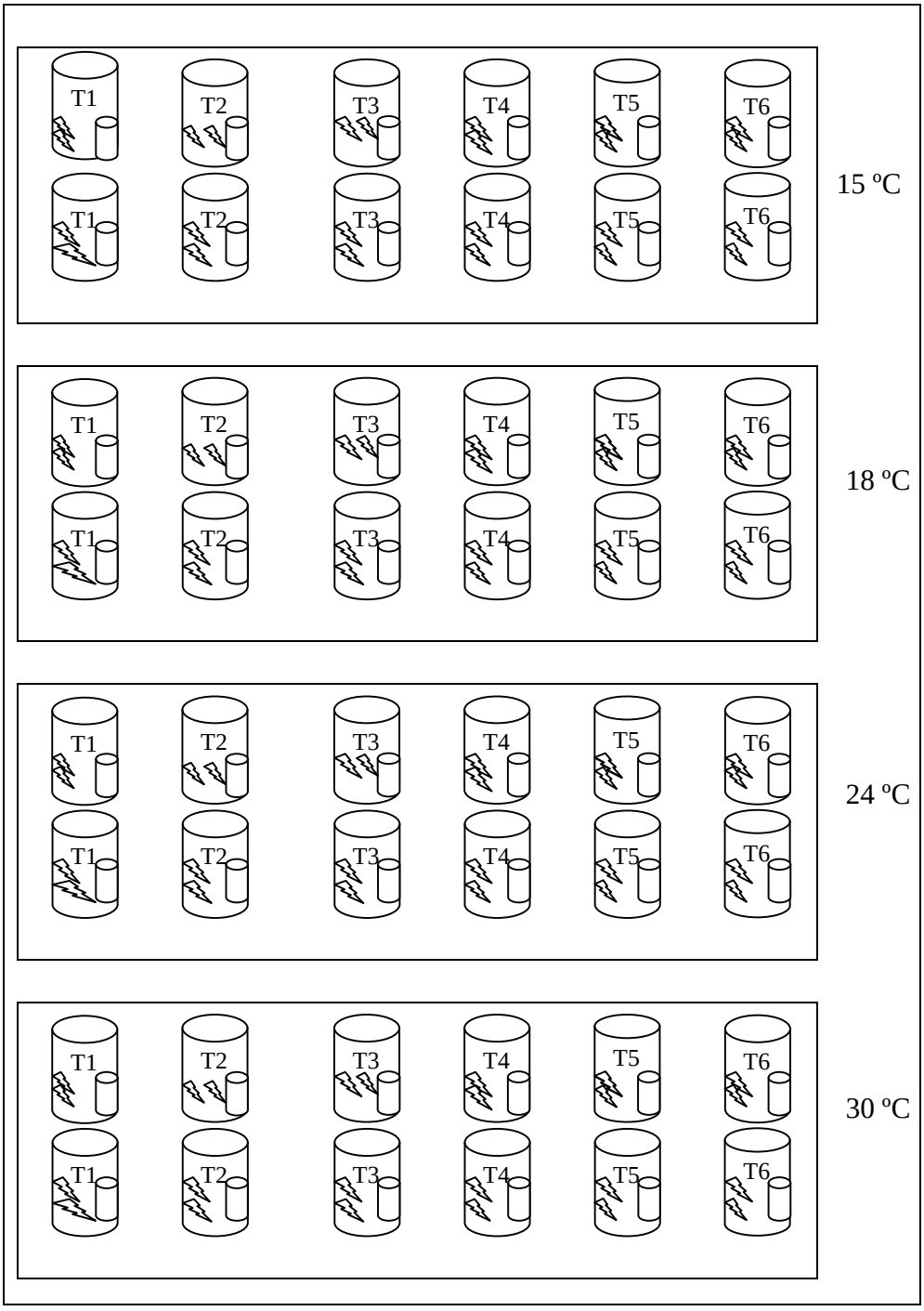


Figure 3.3 Experimental design for determination of mass loss and carbon dioxide evolution of needles after incubation periods in each site.

3.6 Elemental analysis

Subsamples of the litter from each study plot were oven dried at 65 ºC for 72 hours and ground in a laboratory mill (Retsch [Haan, Germany] ZM 100) to pass through 0.5 mm screen. Total N and C concentrations were measured using freshly collected litter samples and samples after 16 weeks of incubation. Analyses were done at Bem laboratory in Cape Town, South Africa using a Leco Truspec CN analyzer.

3.7 Calculations and statistical analyses

- Decomposition rates (k) were calculated by fitting the observed data to the single exponential model proposed by Olson (1963): $X_t=X_oe^{-kt}$ where X_t and X_o are the litter mass at the times t and 0 (initial), respectively; k is the decomposition constant.
- Four-way ANOVAs were used to test for statistical ($p<0.05$) differences in CO_2 evolution and mass loss based on temperature, fertilizer treatment, site and duration of incubation.
- Three-way ANOVAs were used to test for statistical ($p<0.05$) differences in CO_2 evolution and mass loss based on temperature, fertilizer treatment and site at individual incubation periods.
- A three-way ANOVA was used to test for statistical differences in N (%) concentration in the needle litter that was incubating for 16 weeks based on temperature, fertilizer treatment and site.
- Two-way ANOVAs were used to test for statistical differences in C:N and N (%) concentrations of the needle litter with sites and fertilization level as main factors.
- Tukey's method was used for a pair-wise comparison between means.
- Pearson correlation coefficients were used to examine the relationships between mass loss, CO_2 evolution, decomposition rate constant and litter chemistry.
- Correlation between temperature and N (%) accumulation was examined using the Pearson correlation coefficient.

All statistical analyses were performed using R (a statistical software version R 2.4.1, 2006).

Chapter 4- Results

4.1 Litter chemistry at the time of sampling

The total N content of the litter ranged from 1.20% at Elandshoogte to 1.60% at Mooifontein, and 1.38% at Mamre.

Table 4.1 Initial nitrogen and carbon concentration (%) of unfertilized (control) and combined fertilized plots at three sites in Ngodwana plantation in Mpumalanga. Data of unfertilized plots are means of two replicates and those of fertilized plots, 10 replicates (± SE)

	Elandshoogte		**Mamre**		**Mooifontein**	
	Control	+N	Control	+N	Control	+N
N (%)	1.20±0.08[a]	1.28±0.01[a]	1.38±0.03[a]	1.41±0.01[a]	1.52±0.03[b]	1.60±0.06[b]
C (%)	69.4±0.4[a]	68.8±0.18[a]	68.7±0.2[a]	68.7±0.2[a]	69.0±0.2[a]	65.3±2.82[a]
C:N	52.7±2.70[a]	50.3±1.24[a]	57.3±4.29[a]	50.9±0.83[a]	46.7±1.13[b]	41.6±2.09[b]

Different letters in a row indicate significant differences at $p<0.010$ (N %) and $p<0.001$ (C:N). Two-way ANOVA showed no interactive effects.

Generally, the N concentrations in the litter differed significantly among sites with Elandshoogte having the lowest concentrations and Mooifontein the highest (Tables 4.1 and 4.2). Litter N concentrations increased in all the sites upon additional N supply with the highest relative response in Elandshoogte (6.67%), 5.27% for Mooifontein and lowest in Mamre (2.17%).The highest % increase was recorded for the site with the lowest N concentration for the control although these increases were not statistically significant. The form and amount of fertilizer applied did not affect litter N concentration at each site however; when the analysis for the three site were pooled together the form and time of fertilizer application had a significant effect. The highest N (%) was recorded for the sites that were treated with Urea (46) at 11 and 13 years while the control recorded the lowest N concentration. The litter N concentration also varied with time of the fertilizer

application where litter collected from sites fertilized at 13 years recorded high N concentration than that of 11 years (Figure 4.1). Litter C concentration only varied from 68.2% to 69.6% and was not significant and therefore changes in litter C:N ratio mirrored those for litter N concentrations. There was no significant interaction between site and fertilization on litter chemistry assessed (Appendix 1).

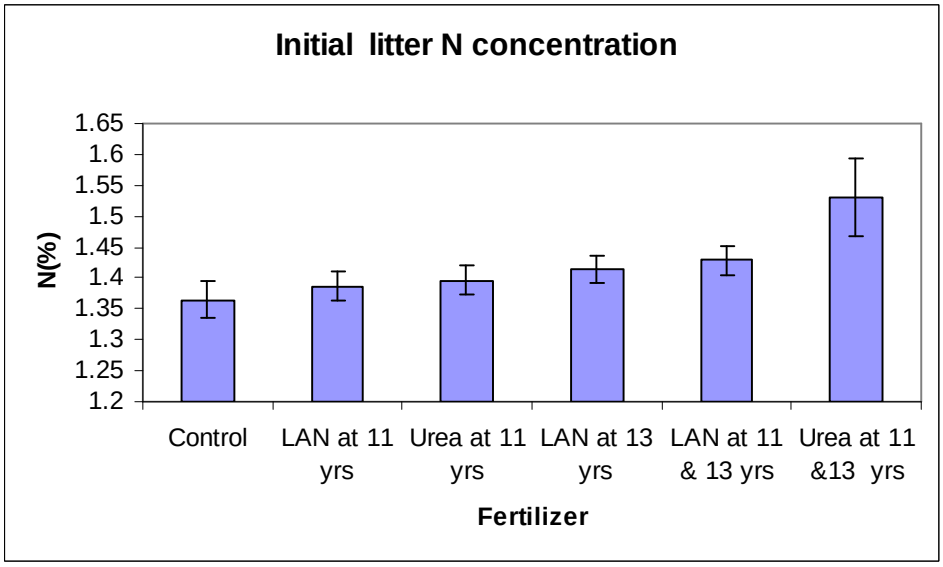


Figure 4.1 Initial nitrogen concentration of sites categorized according to the form of fertilizer and time since application. Values are the means±SE derived from pooled data from three sites.

4.2 Litter chemistry changes over the 16 weeks of incubation

For all but 11 of 144 litter samples that were incubated at different temperatures for 16 weeks, there was an increase in N (%) concentration (Table 4.3). Paired student's t-test result showed that there were significant differences between N concentration of the initial litter and after incubation for 16 weeks ($p<0.001$).

Table 4.2 Nitrogen (N) concentrations (%) of litter from unfertilized (control) and fertilized sites before and after incubation for 16 weeks at four temperatures.

Initial	Site	Temperature	control	LAN (28) at 11 yrs	Urea (46) at 11 yrs	LAN (28) at 13 yrs	LAN (28) at 11 & 13 yrs	Urea (46) at 11 & 13 yrs
	Elandshoogte	15 ^{0}C	1.32±0.08	1.38±0.05	1.29±0.09	1.4±0.24	1.45±0.08	1.38±0.05
		18 ^{0}C	1.32±0.08	1.38±0.05	1.29±0.09	1.4±0.24	1.45±0.08	1.38±0.05
		24 ^{0}C	1.32±0.08	1.38±0.05	1.29±0.09	1.4±0.24	1.45±0.08	1.38±0.05
		30 ^{0}C	1.32±0.08	1.38±0.05	1.29±0.09	1.4±0.24	1.45±0.08	1.38±0.05
	Mamre	15 ^{0}C	1.21±0.12	1.35±0.09	1.39±0.18	1.31±0.08	1.37±0.04	1.35±0.05
		18 ^{0}C	1.21±0.12	1.35±0.09	1.39±0.18	1.31±0.08	1.37±0.04	1.35±0.05
		24 ^{0}C	1.21±0.12	1.35±0.09	1.39±0.18	1.31±0.08	1.37±0.04	1.35±0.05
		30 ^{0}C	1.21±0.12	1.35±0.09	1.39±0.18	1.31±0.08	1.37±0.04	1.35±0.05
	Mooifontein	15 ^{0}C	1.48±0.06	1.48±0.01	1.57±0.04	1.84±0.47	1.52±0.04	1.52±0.04
		18 ^{0}C	1.48±0.06	1.48±0.01	1.57±0.04	1.84±0.47	1.52±0.04	1.52±0.04
		24 ^{0}C	1.48±0.06	1.48±0.01	1.57±0.04	1.84±0.47	1.52±0.04	1.52±0.04
		30 ^{0}C	1.48±0.06	1.48±0.01	1.57±0.04	1.84±0.47	1.52±0.04	1.52±0.04
16 weeks	**Elandshoogte**	15 ^{0}C	1.7±0.01	1.54±0.13	1.47±0.10	1.72±0.11	1.92±0.08	1.3±0.10
		18 ^{0}C	1.71±0.05	1.49±0.05	1.93±0.19	1.65±0.17	1.69±0.08	1.88±0.19
		24 ^{0}C	1.97±0.15	1.64±0.21	1.71±0.45	2.08±0.59	1.55±0.08	1.66±0.07
		30 ^{0}C	1.64±0.09	1.83±0.01	1.84±0.05	1.79±0.37	1.79±0.08	1.82±0.14
	Mamre	15 ^{0}C	1.54±0.08	1.57±0.25	1.50±0.21	1.55±0.08	1.75±0.10	1.39±0.16
		18 ^{0}C	1.56±0.08	1.42±0.16	1.71±0.04	1.58±0.13	1.76±0.04	1.47±0.11
		24 ^{0}C	1.46±0.11	1.59±0.11	1.59±0.22	1.51±0.09	1.72±0.16	1.46±0.04
		30 ^{0}C	1.82±0.14	1.32±0.50	1.59±0.01	1.83±0.10	1.63±0.15	1.65±0.16
	Mooifontein	15 ^{0}C	1.90±0.19	1.98±0.04	1.77±0.00	1.68±0.27	1.81±0.12	1.76±0.08
		18 ^{0}C	1.88±0.28	1.85±0.07	1.85±0.00	2.07±0.35	1.92±0.00	2.11±0.06
		24 ^{0}C	2.16±0.53	1.99±0.25	1.80±0.18	2.25±0.02	2.04±0.10	2.06±0.01
		30 ^{0}C	2.25±0.03	1.79±0.03	1.89±0.50	2.07±0.24	2.17±0.28	2.15±0.03

Three-way ANOVA demonstrated that nitrogen accumulation depended significantly on temperature (p=0.0082), site (p=0.0032) and fertilization (p=0.0291) while interactions were not significant (Appendix 2). For temperature this variation was attributable to differences between 24 ºC and 15 ºC (p=0.0490) and 30 ºC and 15 ºC (p=0.0062); for fertilization the variation was attributable to differences between unfertilized and LAN

(28) 11 yrs plots (p=0.0226) and for site the variation was attributable only to differences between Mooifontein and Mamre (p=0.0034).

Table 4.3 Carbon:nitrogen (C:N) ratio of the litter from unfertilized (control) and fertilized sites before and after incubation for 16 weeks at four temperatures. Values are means ± SD.

Initial	Site	Temperature	control	LAN (28) at 11 yrs	Urea (46) at 11 yrs	LAN (28) at 13 yrs	LAN (28) at 11 & 13 yrs	Urea (46) at 11 & 13 yrs
	Elandshoogte	15 ^{0}C	47.8±7.87	46.8±8.75	53.5±1.88	52.6±2.04	52.5±1.91	52.7±2.08
		18 ^{0}C	47.8±7.87	46.8±8.75	53.5±1.88	52.6±2.04	52.5±1.91	52.7±2.08
		24 ^{0}C	47.8±7.87	46.8±8.75	53.5±1.88	52.6±2.04	52.5±1.91	52.7±2.08
		30 ^{0}C	47.8±7.87	46.8±8.75	53.5±1.88	52.6±2.04	52.5±1.91	52.7±2.08
	Mamre	15 ^{0}C	49.9±1.48	49.5±0.96	49.4±0.65	48.9±1.37	48.3±1.81	47.8±1.96
		18 ^{0}C	49.9±1.48	49.5±0.96	49.4±0.65	48.9±1.37	48.3±1.81	47.8±1.96
		24 ^{0}C	49.9±1.48	49.5±0.96	49.4±0.65	48.9±1.37	48.3±1.81	47.8±1.96
		30 ^{0}C	49.9±1.48	49.5±0.96	49.4±0.65	48.9±1.37	48.3±1.81	47.8±1.96
	Mooifontein	15 ^{0}C	45.9±0.79	45.9±0.74	45.6±0.73	45.4±1.19	44.9±1.52	39.1±9.58
		18 ^{0}C	45.9±0.79	45.9±0.74	45.6±0.73	45.4±1.19	44.9±1.52	39.1±9.58
		24 ^{0}C	45.9±0.79	45.9±0.74	45.6±0.73	45.4±1.19	44.9±1.52	39.1±9.58
		30 ^{0}C	45.9±0.79	45.9±0.74	45.6±0.73	45.4±1.19	44.9±1.52	39.1±9.58
16 weeks	**Elandshoogte**	15 ^{0}C	28.8±0.16	31.5±1.64	33.1±2.08	28.6±2.13	25.6±0.95	37.3±3.00
		18 ^{0}C	28.5±0.91	32.7±1.95	25.3±2.36	30.1±2.79	29±1.72	25.8±3.27
		24 ^{0}C	24.9±1.84	30.2±4.21	29.6±7.93	24.4±6.87	31.7±1.50	29.4±1.08
		30 ^{0}C	29.8±1.54	27.5±5.57	26.9±0.61	28.2±5.43	27.4±1.43	27.1±1.99
	Mamre	15 ^{0}C	31.9±1.34	31.5±5.24	32.4±4.06	31.5±1.54	28±1.39	35.1±4.17
		18 ^{0}C	31.4±2.16	34.4±3.76	28.5±0.43	30.7±2.57	27.6±0.59	33.1±2.54
		24 ^{0}C	34.1±2.19	30.4±1.90	30.9±4.35	32.5±1.94	28.8±2.44	33.5±1.25
		30 ^{0}C	26.4±0.17	39.9±14.9	30.6±0.18	27.1±1.66	30.2±2.45	30±2.18
	Mooifontein	15 ^{0}C	26.4±1.43	24.8±0.23	27.2±0.00	29.5±3.70	27.5±1.21	27.7±0.62
		18 ^{0}C	26.7±3.19	25.8±0.99	26±0.00	23.9±3.46	26.3±0.00	23.6±0.51
		24 ^{0}C	23.7±4.71	24.7±2.45	27±1.83	22.2±0.37	24.5±1.60	24±0.88
		30 ^{0}C	22±0.04	27.4±1.14	27±6.88	24.2±3.23	23.5±3.35	23.3±1.23

C:N ratio depended significantly on temperature ($p=0.03252$) and site ($p<0.01$) while fertilization and interactions were not significant (Appendix 3). The paired student's t-test showed that in all cases there were significant differences between the initial and final C:N ratio of the litter ($p<0.001$).

For temperature this variation in C:N was attributable to differences between 30 ºC and 15 ºC ($p=0.0339$) whereas site variation was due to differences between all three sites with Mooifontein having the lowest and Mamre the highest.

4.3 Rate of CO_2 production from decomposing litter

Figure 4.2 shows patterns of CO_2 emission from litter from the three sites incubated for periods up to 16 weeks at four temperatures. Carbon dioxide produced varied significantly with temperature ($p<0.001$), fertilization ($p<0.001$) and duration of incubation ($p<0.001$) and there were significant interactions between all factors except site:time (Appendix 4).

Temperature accounted for much of the variability of CO_2 produced at all weeks except for week 6 where the fertilization effect was significant (Appendix 5). However, significant two-and three-way interactions on individual weeks indicated that the effect of temperature was frequently associated with fertilization or sites.

Variation in CO_2 emissions across the four incubation periods is mostly explained by temperature implying that temperature plays a dominant role in decomposition of the litter. The patterns shown by individual variables are described below.

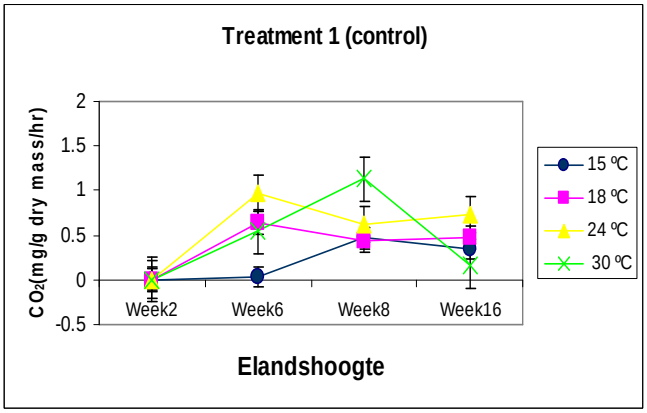
4.3.1 Temperature effect

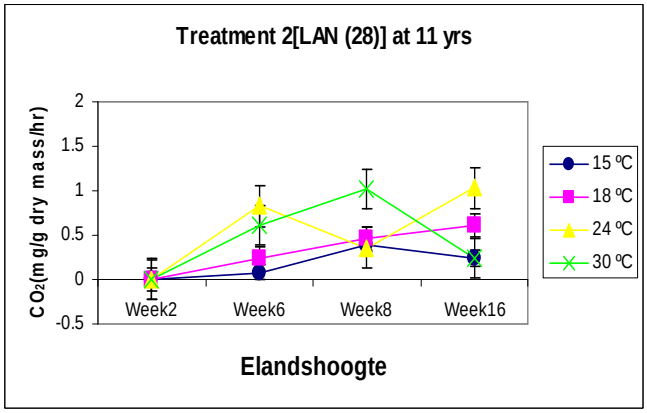
Across all the three sites (Elandshoogte, Mamre and Mooifontein) the amount of CO_2 produced was generally higher for temperatures 24 ºC and 30 ºC compared to 15 ºC and

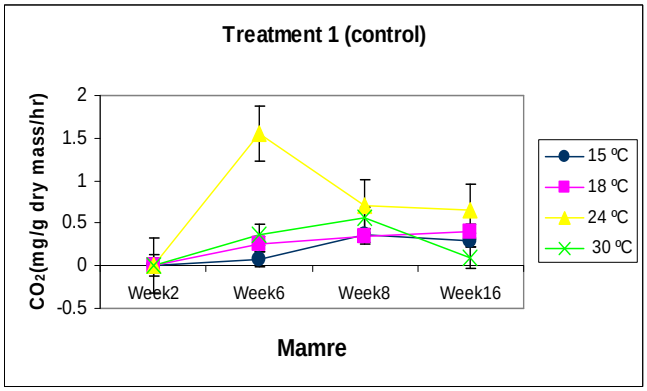
18 ºC. Carbon dioxide produced by samples incubated at 30 ºC in all the sites continued to increase from week 2 until week 8 and sharply declined at 16 weeks (Figure 4).

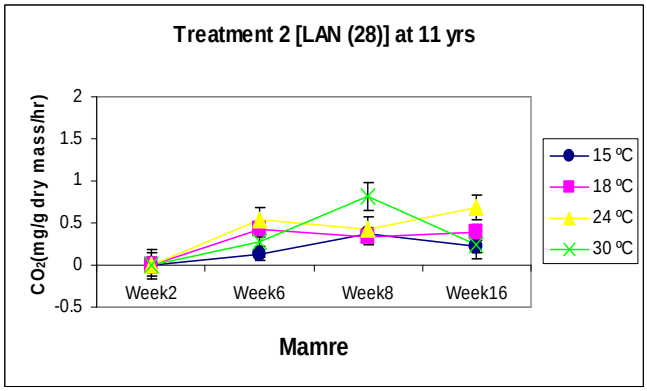
There was a clear difference between CO_2 produced at 15 ºC and 18 ºC ($p<0.001$) with 87% of the data showing 18 ºC yielding more CO_2 than 15 ºC. The amount of CO_2 produced at high temperatures especially 30 ºC was significantly higher than that of 15 ºC and 18 ºC.

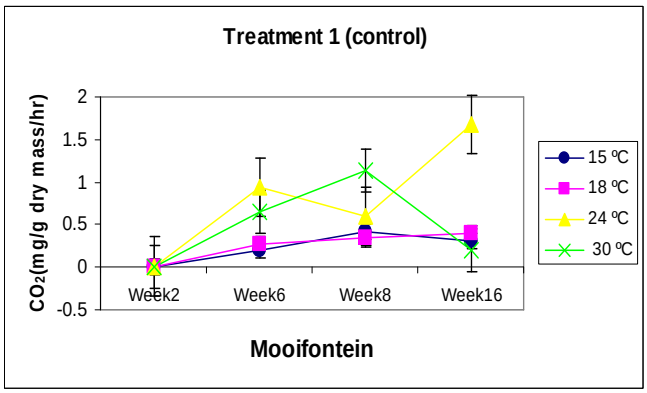
Figure 4.2 CO_2 emissions of litter from three unfertilized (control) and fertilized sites incubated for up to 16 weeks at four temperatures.

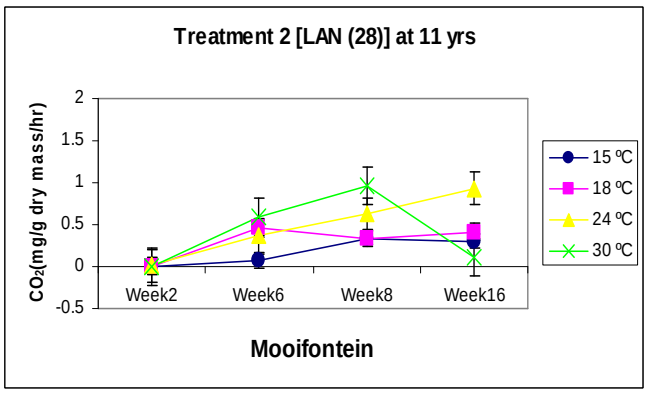


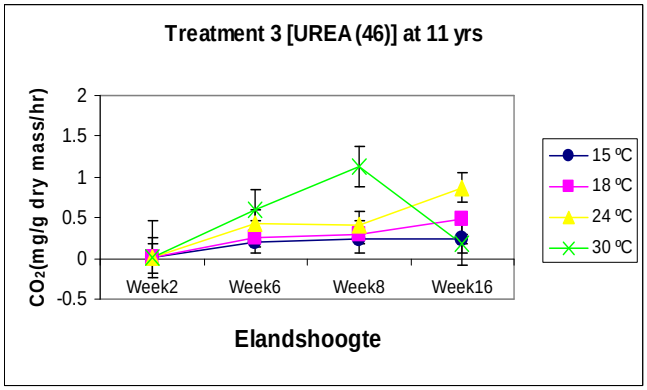


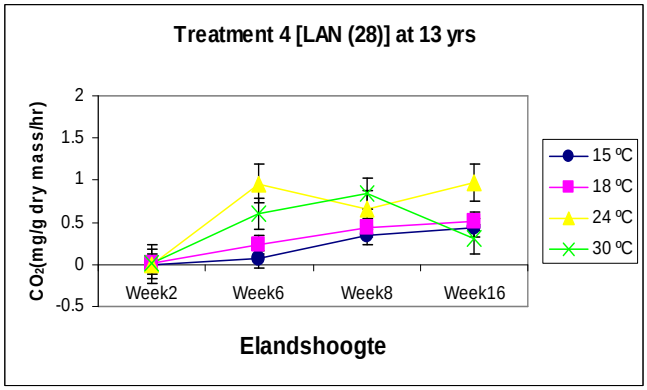


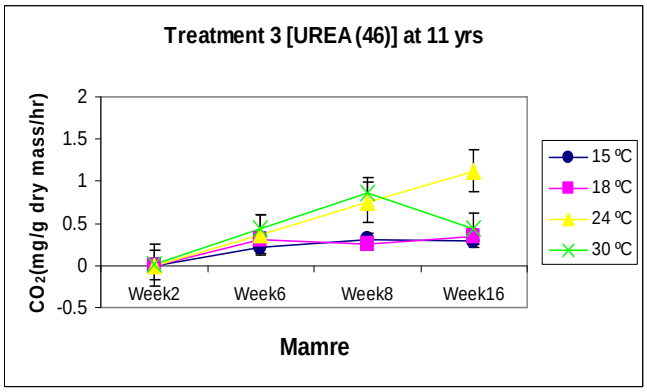


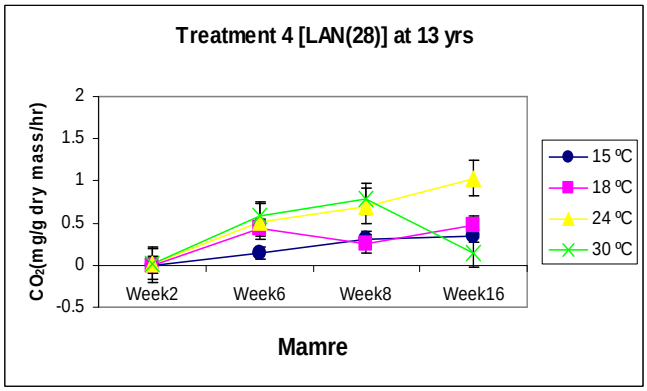


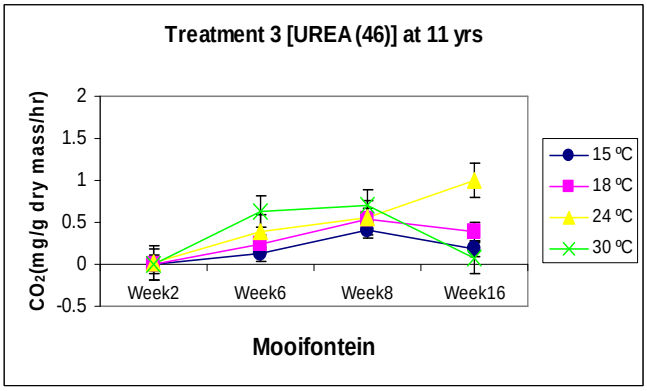


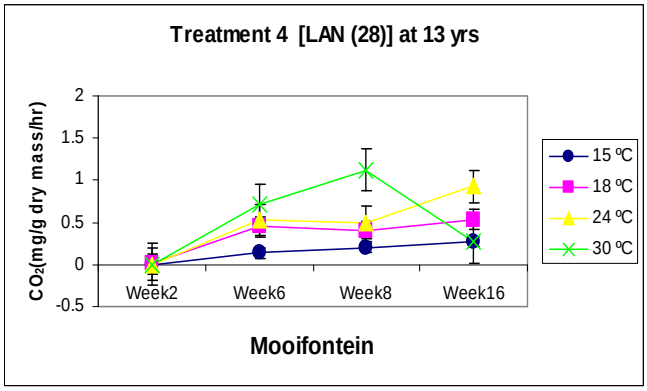

Treatment 3 [UREA (46)] at 11 yrs
CO2(mg/g dry mass/hr)
2
1.5
1
0.5
0
-0.5
Week2
Week6
Week8
Week16
15 ºC
18 ºC
24 ºC
30 ºC
Elandshoogte

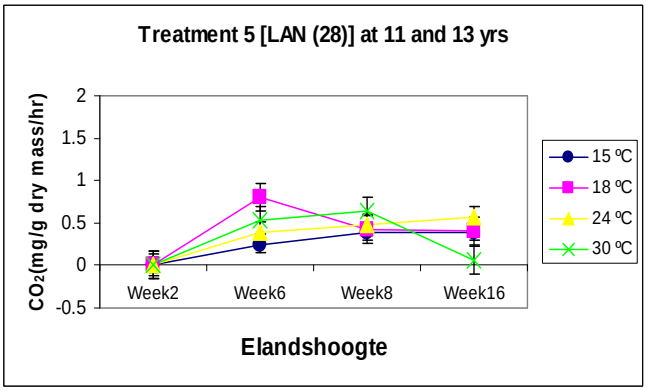

Treatment 4 [LAN (28)] at 13 yrs
CO2(mg/g dry mass/hr)
2
1.5
1
0.5
0
-0.5
Week2
Week6
Week8
Week16
15 ºC
18 ºC
24 ºC
30 ºC
Elandshoogte

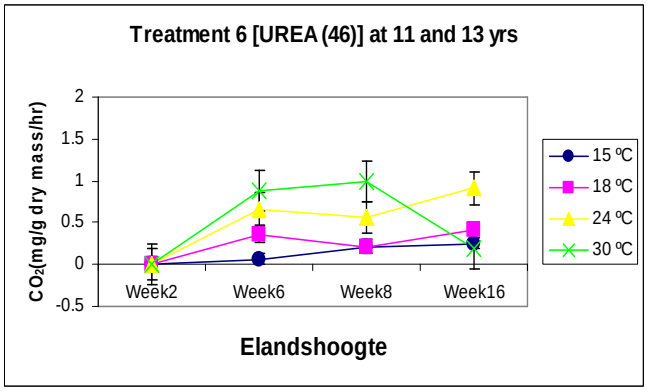

Treatment 3 [UREA (46)] at 11 yrs
CO2(mg/g dry mass/hr)
2
1.5
1
0.5
0
-0.5
Week2
Week6
Week8
Week16
15 ºC
18 ºC
24 ºC
30 ºC
Mamre

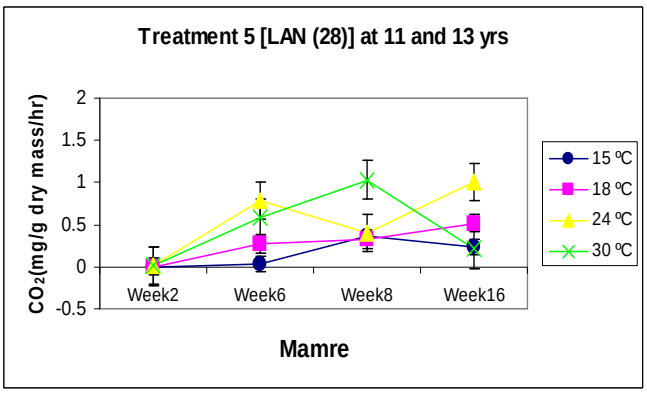

Treatment 4 [LAN(28)] at 13 yrs
CO2(mg/g dry mass/hr)
2
1.5
1
0.5
0
-0.5
Week2
Week6
Week8
Week16
15 ºC
18 ºC
24 ºC
30 ºC
Mamre

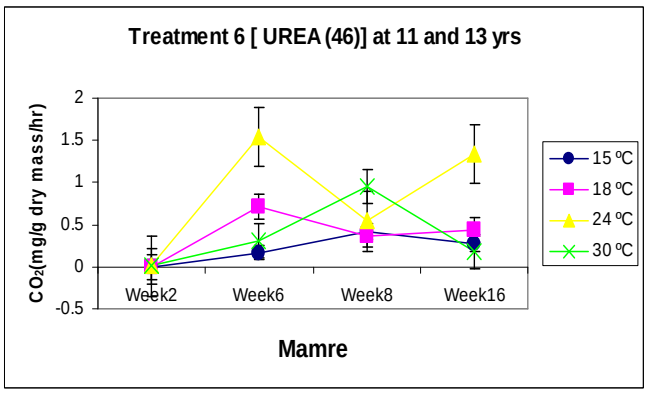

Treatment 3 [UREA (46)] at 11 yrs
CO2(mg/g dry mass/hr)
2
1.5
1
0.5
0
-0.5
Week2
Week6
Week8
Week16
15 ºC
18 ºC
24 ºC
30 ºC
Mooifontein

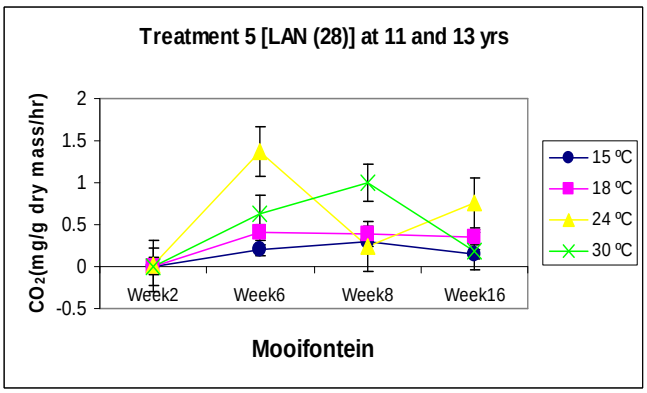

Treatment 4 [LAN (28)] at 13 yrs
CO2(mg/g dry mass/hr)
2
1.5
1
0.5
0
-0.5
Week2
Week6
Week8
Week16
15 ºC
18 ºC
24 ºC
30 ºC
Mooifontein

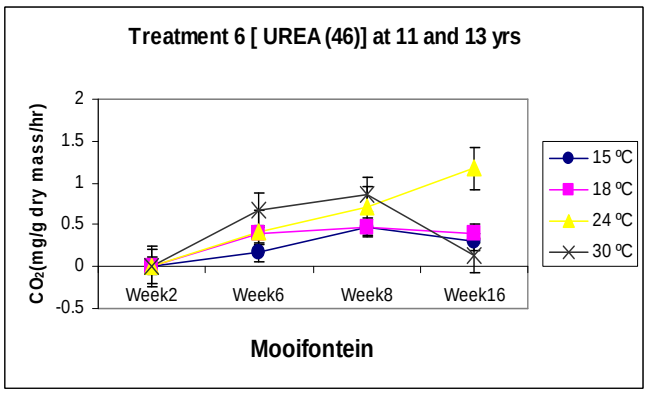

Treatment 5 [LAN (28)] at 11 and 13 yrs
CO2(mg/g dry mass/hr)
2
1.5
1
0.5
0
-0.5
Week2
Week6
Week8
Week16
15 ºC
18 ºC
24 ºC
30 ºC
Elandshoogte


Treatment 6 [UREA (46)] at 11 and 13 yrs
CO2(mg/g dry mass/hr)
2
1.5
1
0.5
0
-0.5
Week2
Week6
Week8
Week16
15 ºC
18 ºC
24 ºC
30 ºC
Elandshoogte


Treatment 5 [LAN (28)] at 11 and 13 yrs
CO2(mg/g dry mass/hr)
2
1.5
1
0.5
0
-0.5
Week2
Week6
Week8
Week16
15 ºC
18 ºC
24 ºC
30 ºC
Mamre


Treatment 6 [UREA (46)] at 11 and 13 yrs
CO2(mg/g dry mass/hr)
2
1.5
1
0.5
0
-0.5
Week2
Week6
Week8
Week16
15 ºC
18 ºC
24 ºC
30 ºC
Mamre


Treatment 5 [LAN (28)] at 11 and 13 yrs
CO2(mg/g dry mass/hr)
2
1.5
1
0.5
0
-0.5
Week2
Week6
Week8
Week16
15 ºC
18 ºC
24 ºC
30 ºC
Mooifontein


Treatment 6 [UREA (46)] at 11 and 13 yrs
CO2(mg/g dry mass/hr)
2
1.5
1
0.5
0
-0.5
Week2
Week6
Week8
Week16
15 ºC
18 ºC
24 ºC
30 ºC
Mooifontein

4.3.2 Fertilizer effect

The amount of CO_2 produced from the litter collected from the study plots in which N fertilizer was applied showed similar trends to the control with respect to temperature. ANOVA results indicate that fertilization is significant ($p=0.0088$). LAN (28) at 11 years, Urea (46) at 11 years and LAN (28) at 13 years were significantly different when compared with the control. The effect of fertilization is masked when the analysis was done at individual incubation periods (appendix.. table 9). However, the fertilization effect was significant at week 6 ($p<0.001$).

4.3.3 Duration (Time) effect

Generally CO_2 was produced continuously and increased during the 16 weeks of measurement yielding a significant effect of time on CO_2 production ($p<0.001$). The increase of the CO_2 produced with time was consistent over the four different temperatures although the litter incubated at 30 ºC recorded a decline after 8 weeks.

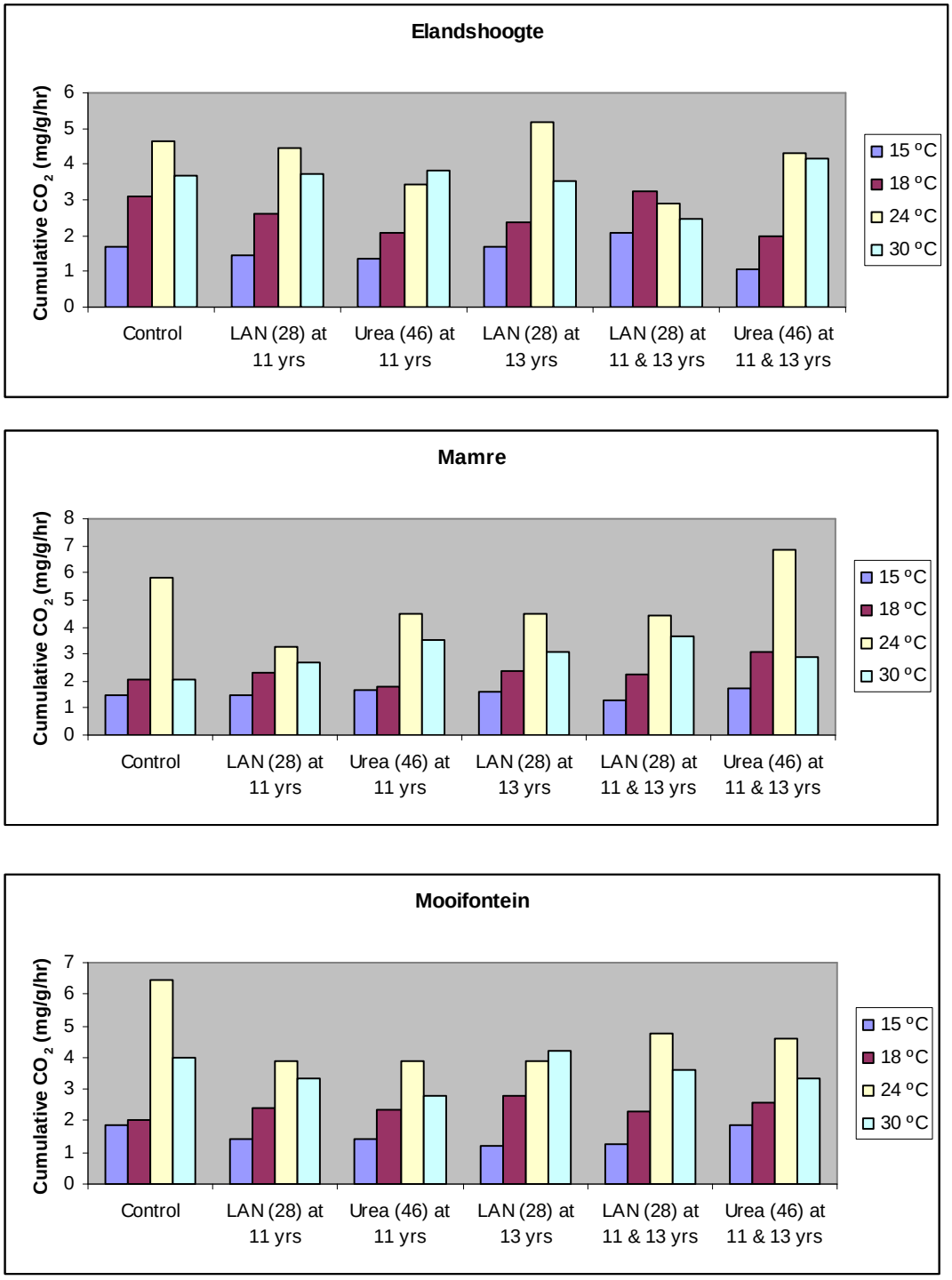


Figure 4.3 Cumulative CO_2 emissions from three unfertilized (control) and fertilized sites incubated for 16 weeks at four temperatures.

In order to teaze out the overall dominant variables influencing CO_2 emission, the total CO_2 emission, the total CO_2 calculated from addition of CO_2 levels at each incubation period was plotted (Figure 4.3). Cumulative CO_2 production (successive additions of CO_2 from week 2 up to week 16) varied significantly with temperature ($p<0.001$), while site and fertilization effects were not significant (Appendix 6). The general trend was for the amount of CO_2 produced increased with an increase in temperature from 15 ºC up to 24 ºC and declined after 24 ºC. Correlation between cumulative CO_2 and temperature was significant ($r=0.6648$, $p<0.001$)**.**

The amount of CO_2 produced was mainly influenced by temperature with high temperature leading to high production of CO_2. The effect of fertilization was not uniform across the weeks and the amount of CO_2 emission from the litter increased with duration of incubation.

4.4 Mass loss

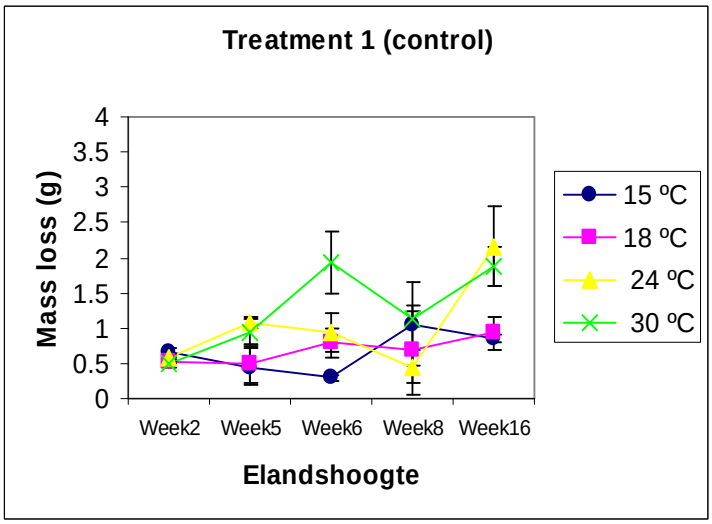
Figure 4.4 shows patterns of mass loss of litter from three sites incubated at four temperatures for periods up to 16 weeks. Mass loss varied significantly with temperature ($p<0.001$), N fertilization ($p<0.001$), site ($p<0.001$) and duration of incubation ($p<0.001$). The higher order interactions (four-way) between all the factors were not significant even though some two-way interactions were significant i.e. temperature:time ($p<0.001$), fertilization:site ($p<0.001$) and time:site ($p=0.004$) (Appendix 7)

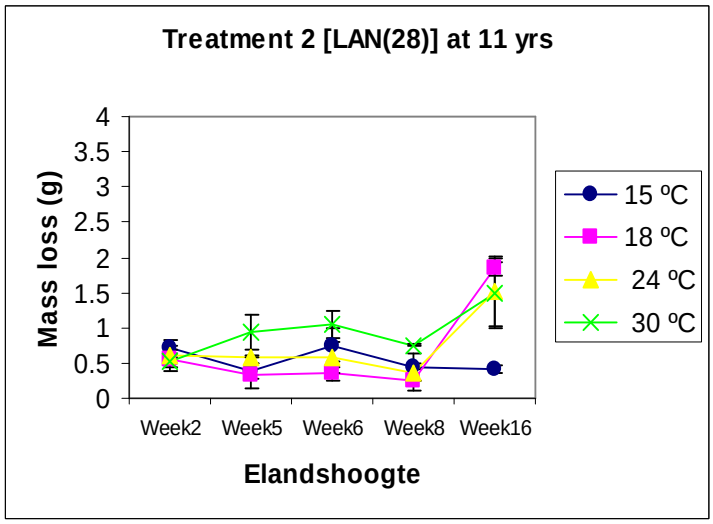
When the analyses were done at each individual week, mass loss was significantly affected by temperature, fertilization and site at all time periods (Appendix 8). Some two-way interactions were also significant i.e. site:fertilization (Appendix 8). The patterns shown by individual variables are described below.

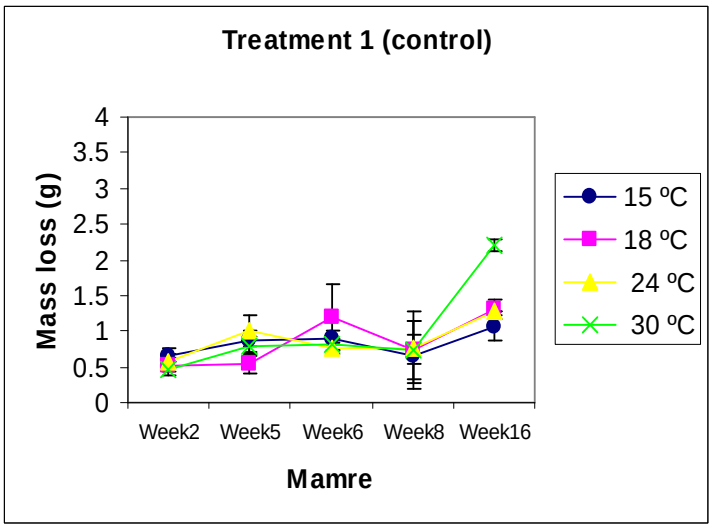
4.4.1 Effect of temperature

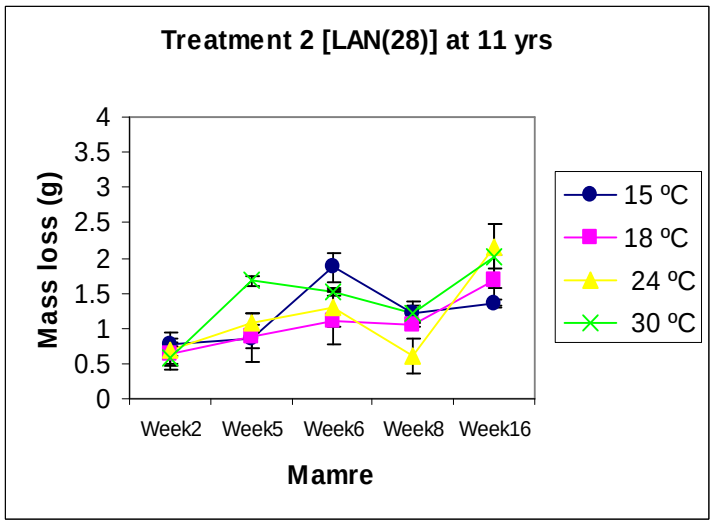
Higher temperatures (24 ºC and 30 ºC) yielded mass loss that was significantly higher to that of 18 ºC and 15 ºC ($p<0.001$). Across all the sites mass loss of litter incubated at 18 ºC is higher than 15 ºC which is confirmed by 65 % of the data showing litter incubated at 18 ºC losing more mass than at 15 ºC ($p<0.001$) (Figure 4.4). Generally litter incubated at 30 ºC lost more mass than that incubated at 15 ºC, 18 ºC and 24 ºC although some exceptions include litter collected from control plot in Mamre at week 6 and that of LAN at 11 yrs. The general trend in figure 4.4 is that increase in temperature result to increase in the amount of mass loss of the litter.

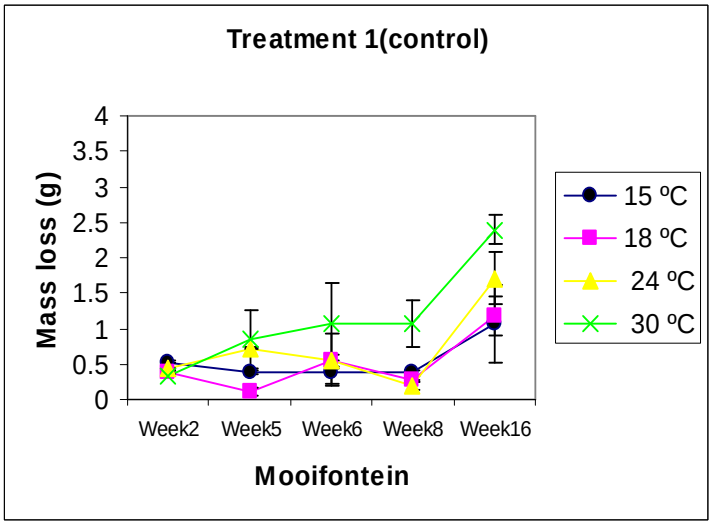
Figure 4.4 Mass loss of litter from three unfertilized (control) and fertilized sites incubated for up to 16 weeks at four temperatures. Values are means ±SEs.

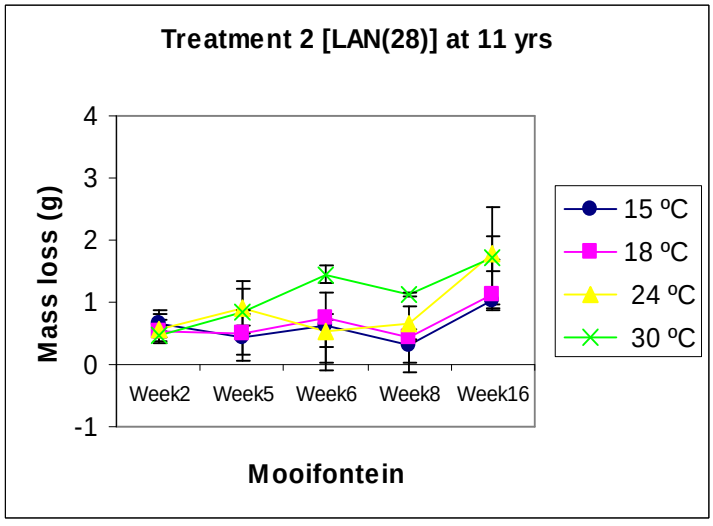


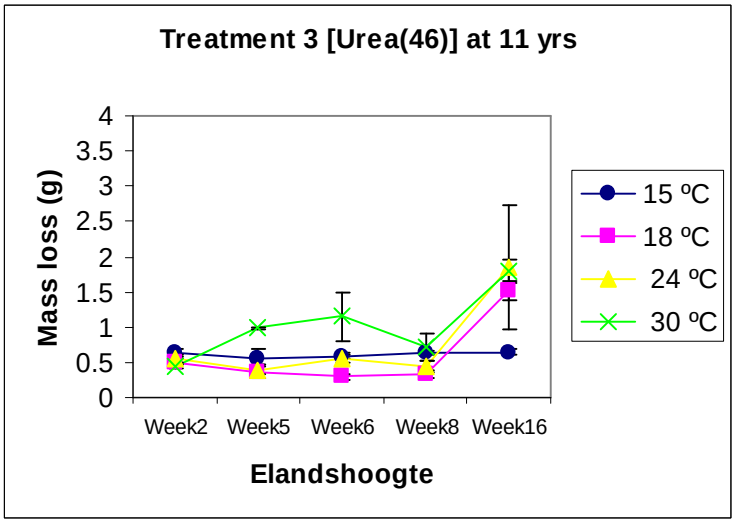


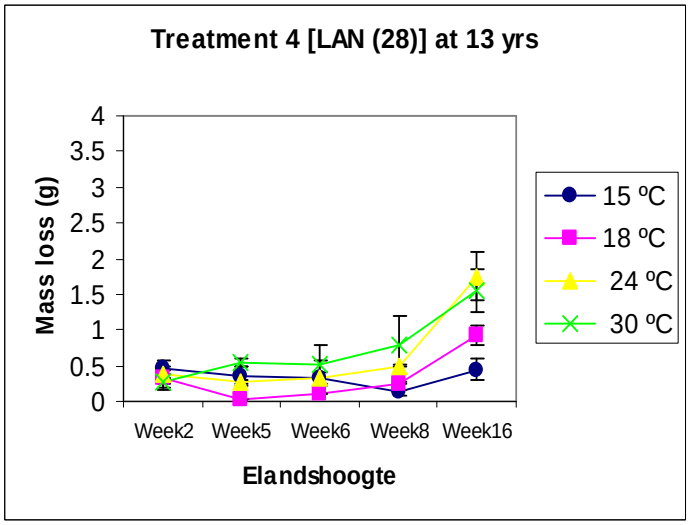


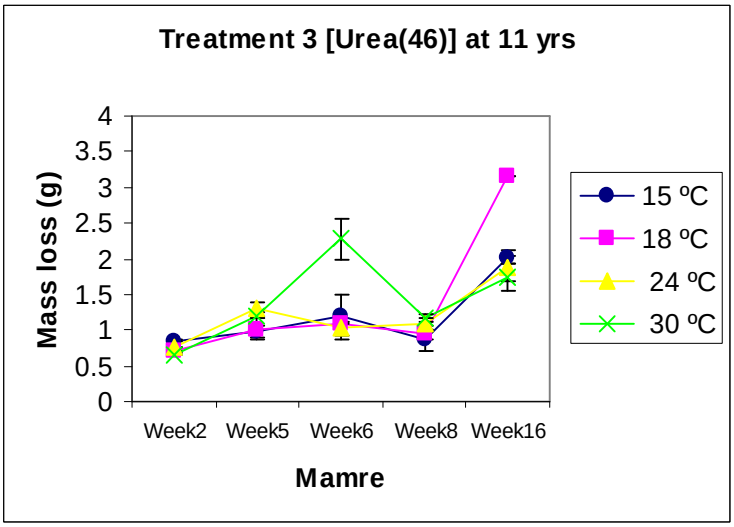


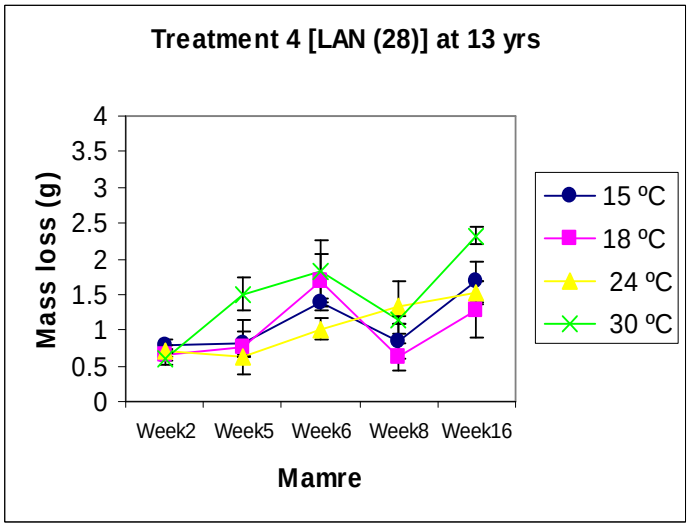


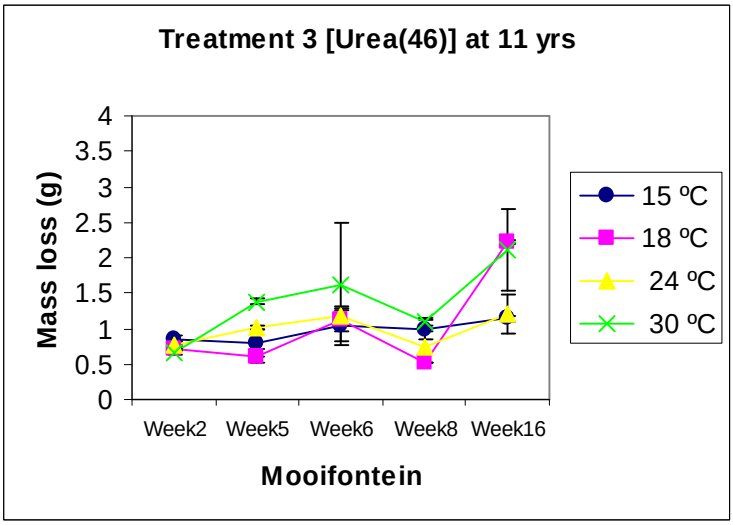


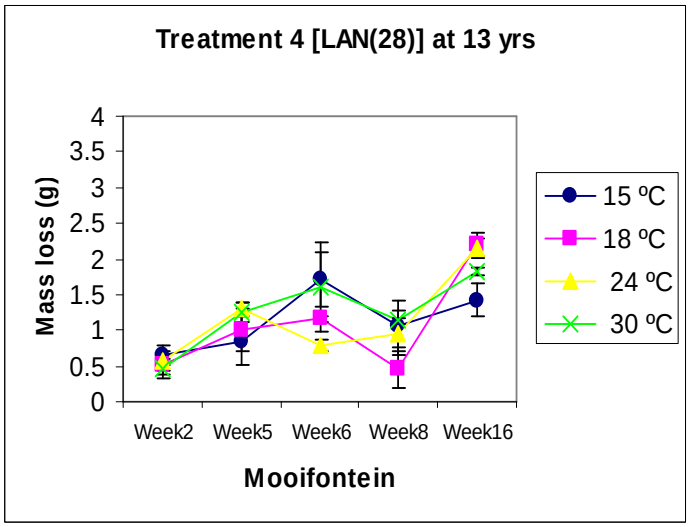

Treatment 3 [Urea(46)] at 11 yrs
Mass loss (g)
4
3.5
3
2.5
2
1.5
1
0.5
0
Week2
Week5
Week6
Week8
Week16
Elandshoogte
15 ºC
18 ºC
24 ºC
30 ºC

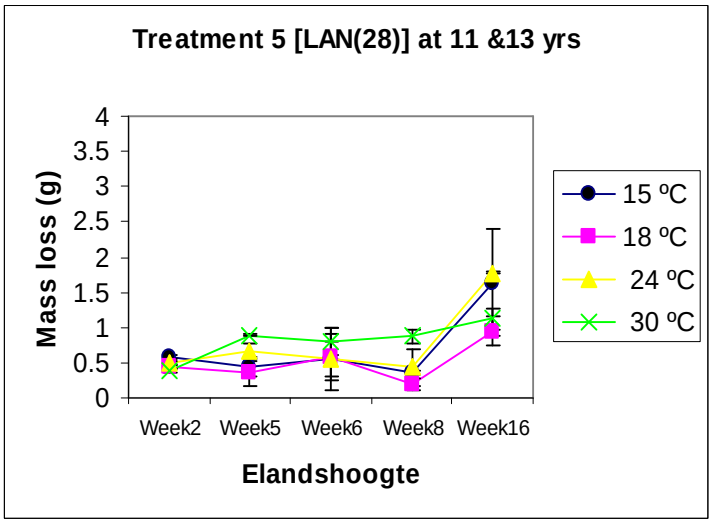

Treatment 4 [LAN (28)] at 13 yrs
Mass loss (g)
4
3.5
3
2.5
2
1.5
1
0.5
0
Week2
Week5
Week6
Week8
Week16
Elandshoogte
15 ºC
18 ºC
24 ºC
30 ºC

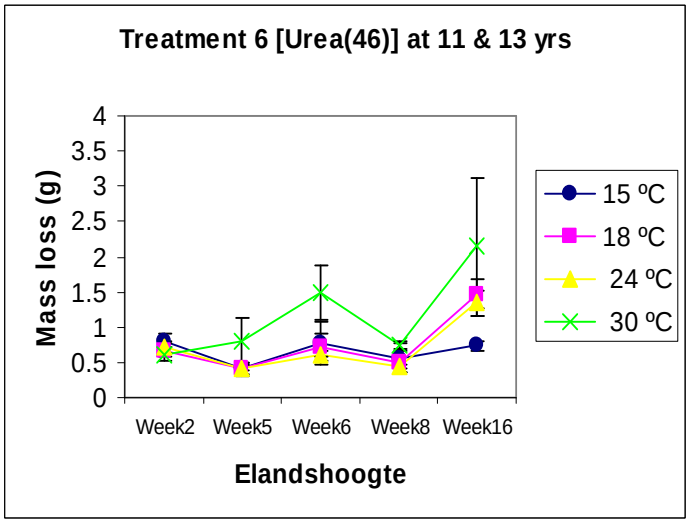

Treatment 3 [Urea(46)] at 11 yrs
Mass loss (g)
4
3.5
3
2.5
2
1.5
1
0.5
0
Week2
Week5
Week6
Week8
Week16
Mamre
15 ºC
18 ºC
24 ºC
30 ºC

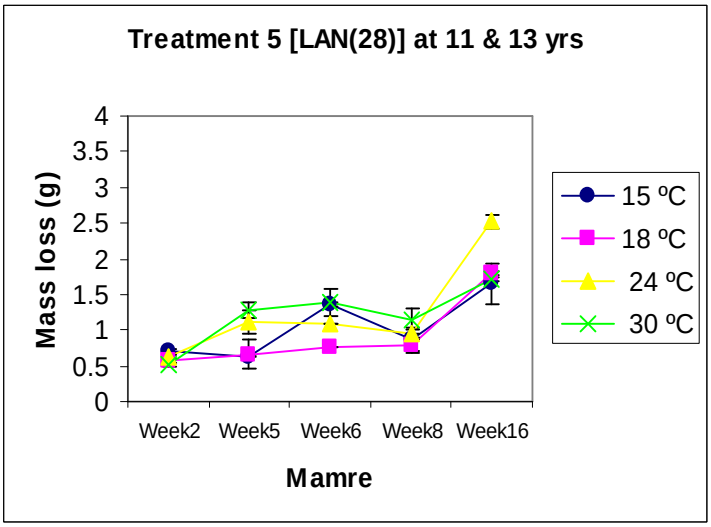

Treatment 4 [LAN (28)] at 13 yrs
Mass loss (g)
4
3.5
3
2.5
2
1.5
1
0.5
0
Week2
Week5
Week6
Week8
Week16
Mamre
15 ºC
18 ºC
24 ºC
30 ºC

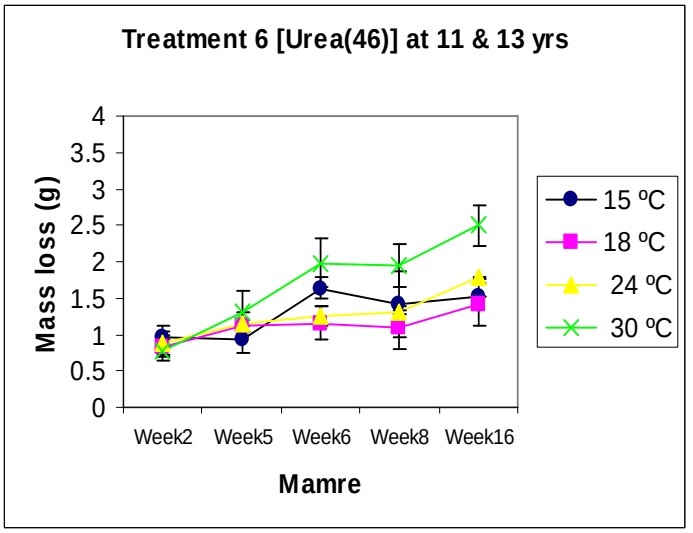

Treatment 3 [Urea(46)] at 11 yrs
Mass loss (g)
4
3.5
3
2.5
2
1.5
1
0.5
0
Week2
Week5
Week6
Week8
Week16
Mooifontein
15 ºC
18 ºC
24 ºC
30 ºC

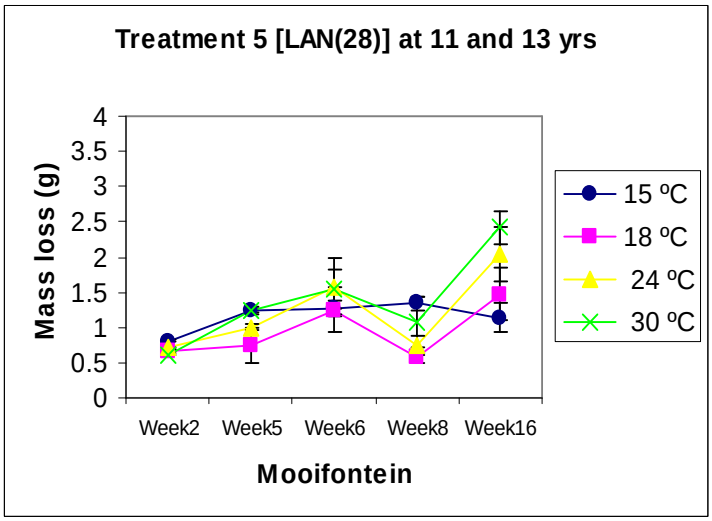

Treatment 4 [LAN(28)] at 13 yrs
Mass loss (g)
4
3.5
3
2.5
2
1.5
1
0.5
0
Week2
Week5
Week6
Week8
Week16
Mooifontein
15 ºC
18 ºC
24 ºC
30 ºC

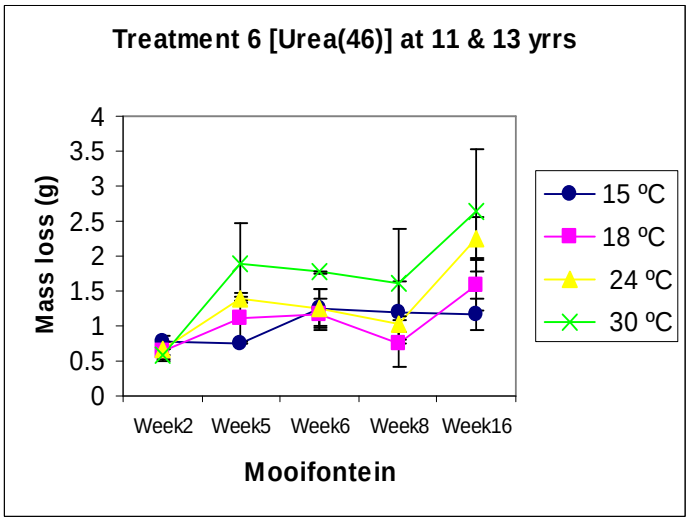

Treatment 5 [LAN(28)] at 11 &13 yrs
Mass loss (g)
4
3.5
3
2.5
2
1.5
1
0.5
0
Week2
Week5
Week6
Week8
Week16
15 ºC
18 ºC
24 ºC
30 ºC
Elandshoogte


Treatment 6 [Urea(46)] at 11 & 13 yrs
Mass loss (g)
4
3.5
3
2.5
2
1.5
1
0.5
0
Week2
Week5
Week6
Week8
Week16
15 ºC
18 ºC
24 ºC
30 ºC
Elandshoogte


Treatment 5 [LAN(28)] at 11 & 13 yrs
Mass loss (g)
4
3.5
3
2.5
2
1.5
1
0.5
0
Week2
Week5
Week6
Week8
Week16
15 ºC
18 ºC
24 ºC
30 ºC
Mamre


Treatment 6 [Urea(46)] at 11 & 13 yrs
Mass loss (g)
4
3.5
3
2.5
2
1.5
1
0.5
0
Week2
Week5
Week6
Week8
Week16
15 ºC
18 ºC
24 ºC
30 ºC
Mamre


Treatment 5 [LAN(28)] at 11 and 13 yrs
Mass loss (g)
4
3.5
3
2.5
2
1.5
1
0.5
0
Week2
Week5
Week6
Week8
Week16
15 ºC
18 ºC
24 ºC
30 ºC
Mooifontein


Treatment 6 [Urea(46)] at 11 & 13 yrrs
Mass loss (g)
4
3.5
3
2.5
2
1.5
1
0.5
0
Week2
Week5
Week6
Week8
Week16
15 ºC
18 ºC
24 ºC
30 ºC
Mooifontein

4.4.2 Fertilizer effect

N fertilization had a significant effect on mass loss ($p<0.001$). However, the pattern was not consistent with some levels of N fertilization showing no effect when compared with the control. For example the mass loss of the plot fertilized with Limestone Ammonium Nitrate (LAN) 28% at 11 years was not significantly different from the control ($p=0.6133$), a similar result was found when samples obtained from the plot fertilized with LAN (28%) at 13 years was compared with the control ($p=0.4436$). However, mass loss of plots fertilized with Urea (46) at 11 years, LAN (28) at 11 and 13 years and Urea (46) at 11 and 13 years showed significant difference from the control ($p<0.012$).

4.4.3 Duration (time) effect

Duration of incubation (time) effect on mass loss was significant ($p<0.001$). Generally mass loss increased as decomposition progressed. The highest mass loss was recorded at 16 weeks while at week two the lowest mass loss was recorded. Some exceptions include litter incubated at 15 ºC at week 8 for LAN at 11 and 13 yrs lost less mass than one at week 6.

4.4.4 Site effect

Site was a significant factor affecting mass loss of the litter ($p<0.001$). Higher mass losses were recorded at the Mooifontein and Mamre sites than at the Elandshoogte site.

In order to confirm the overall dominant variable influencing mass loss, the total mass loss calculated from addition of mass loss levels at each incubation period was plotted (Fig.4.5)

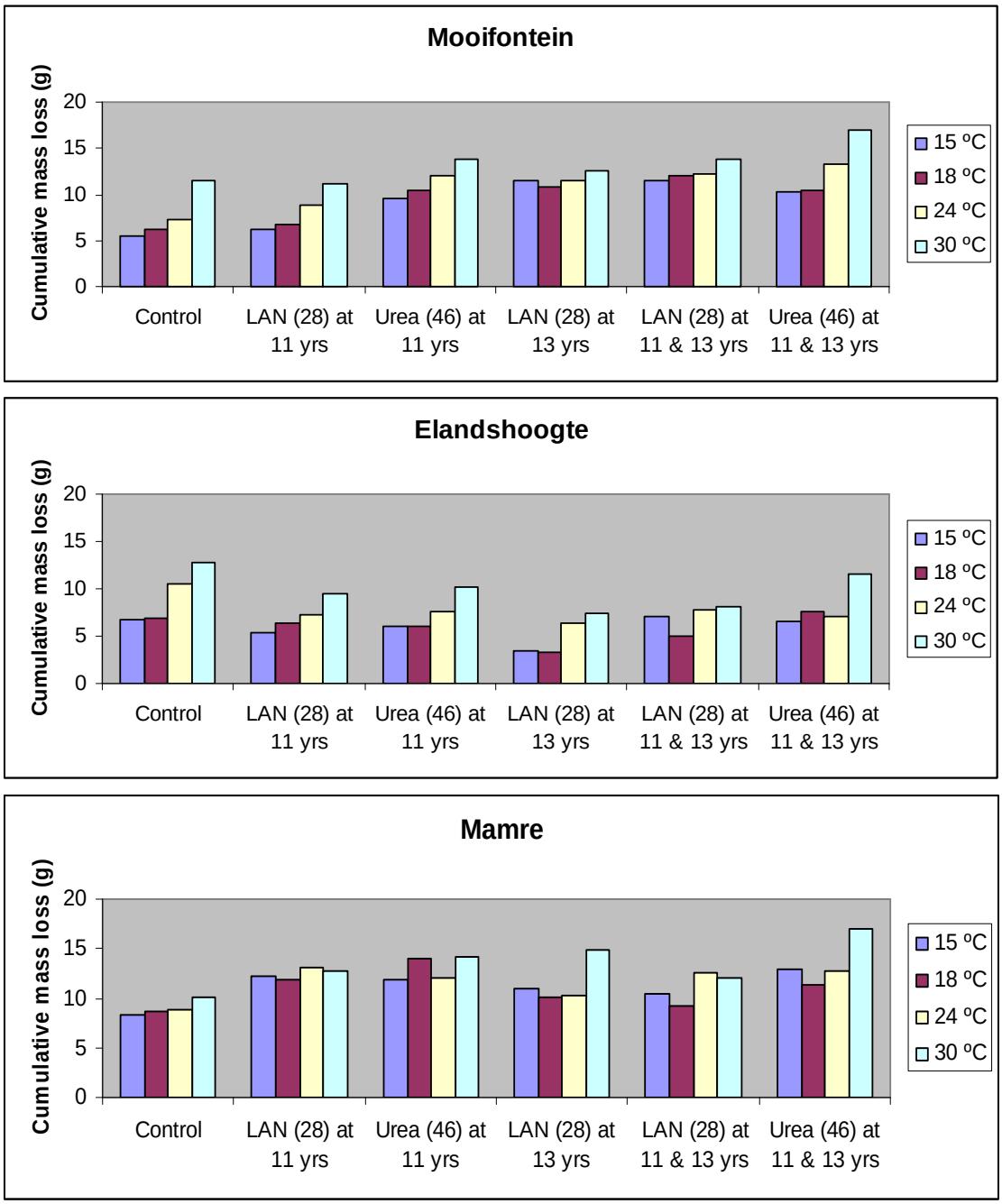


Figure 4.5 Cumulative mass loss of litter from three unfertilized (control) and fertilized sites incubated for 16 weeks at four temperatures.

Cumulative mass loss varied significantly with temperature ($p<0.001$), fertilization ($p=0.0017$) and site ($p<0.001$) (Appendix 9). It is clear from Fig 4.5 that for all treatments higher temperatures resulted in greater mass loss of the litter. The fertilizer effect was significant between plots that were fertilized with Urea (46) at 11 and 13 years and the control ($p<0.001$), LAN (28) at 11 years and Urea (46) at 11 and 13 years ($p<0.0284$).
Cumulative mass loss varied significantly between all the sites; Mamre:Mooifontein ($p=0.0259$), Mamre:Elandshoogte($p<0.001$), Mooifontein:Elandshoogte ($p<0.001$).

4.5 N (%) concentration

Generally mass loss was positively correlated with initial N (%) concentration of the needle litter. However, the correlation was extremely weak ($r=0.07$, $p=0.03$). When tested at each site, mass loss and N (%) correlation was only evident at Mooifontein. The correlation value also diminished as the decomposition progressed: $r=0.1$ after 8 weeks and after 16 weeks dropped to 0.07. However, there was significant correlation between cumulative mass loss and litter nitrogen concentration ($r=0.414$, $p<0.01$) Fig 4.6. Litter cumulative mass loss was positively correlated with the amount of cumulative CO_2 ($r=0.212$, $p=0.0741$) Fig 4.7.

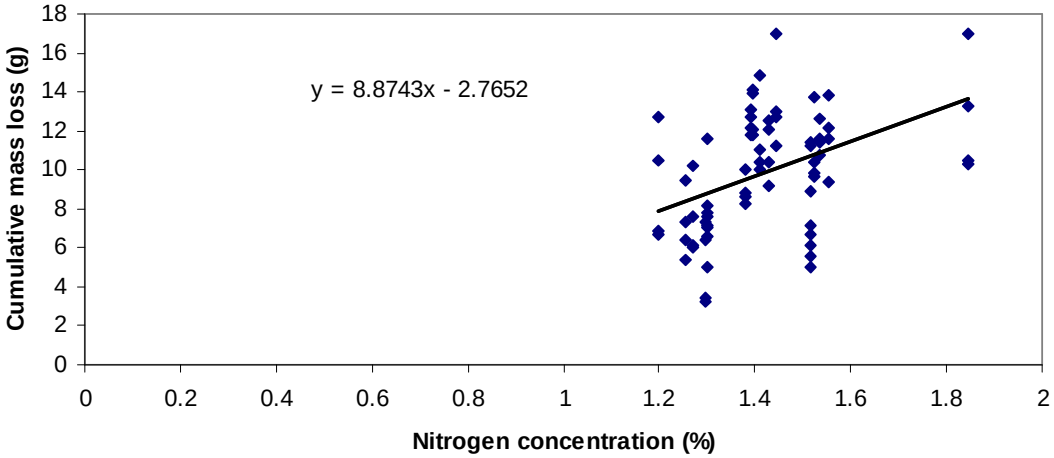


Figure 4.6 Relationship between cumulative mass loss and initial nitrogen concentration of the litter

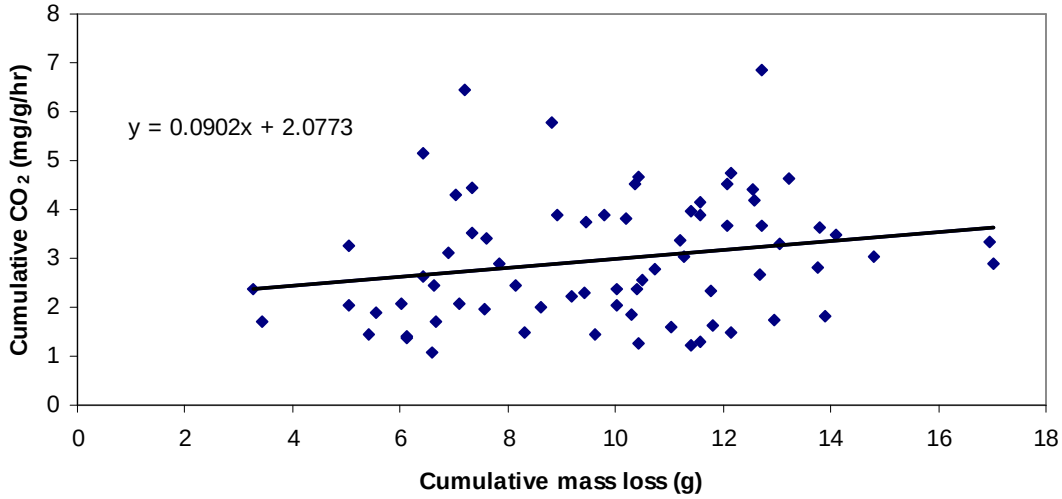


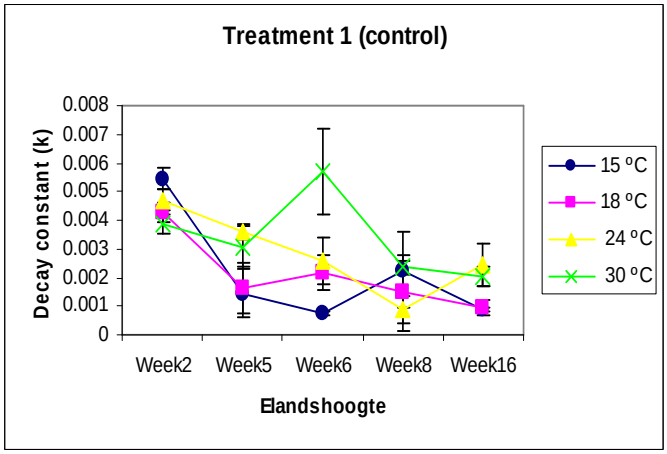
Figure 4.7 Relationship between litter cumulative mass loss and cumulative CO_2

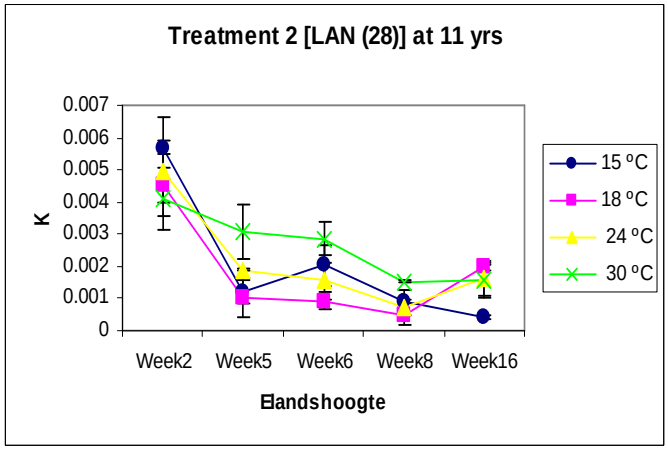
4.5 Decomposition rate constant (k)

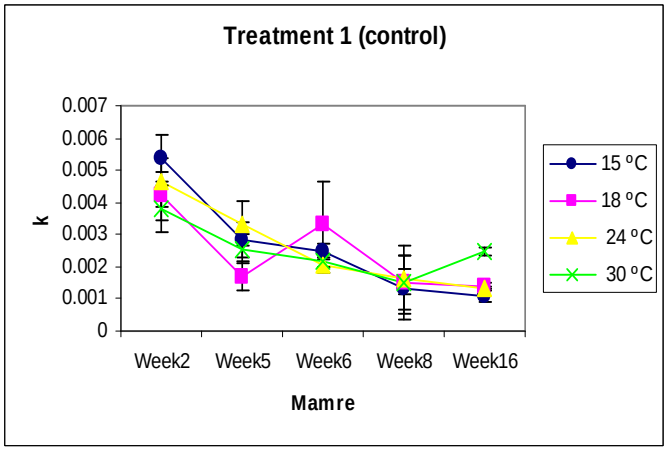
Figure 4.8 shows the decomposition constants of litter from unfertilized and fertilized sites incubated at four temperatures for periods up to 16 weeks. Decomposition constant (k) varied significantly with temperature ($p<0.001$), fertilization ($p<0.001$), site ($p<0.001$) and time ($p<0.001$) with four-way interaction of these factors being not significant. However, several two-way and three-way interactions indicated significant differences (Appendix 10). Compared to litter collected from control plots the k values obtained from N fertilized plots showed no consistent pattern with some fertilization levels being significantly different from the control while others were not (Appendix 10).

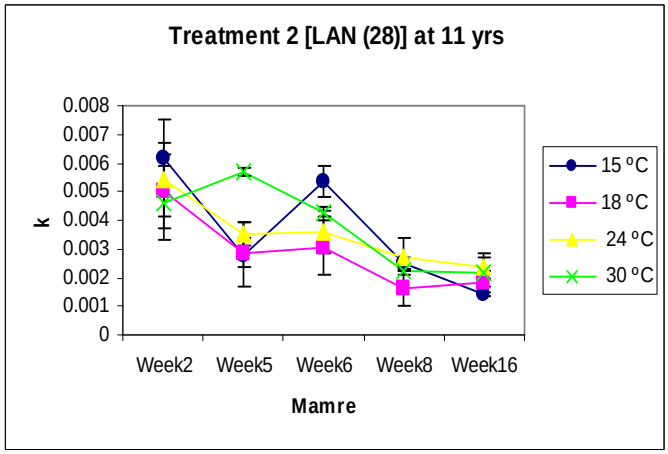
The k values are significantly different between the three sites. Mamre and Mooifontein sites recorded higher k value than Elandshoogte site. The highest k value was recorded at Mamre site. The duration of incubation (week) also affects the k values. There was significant variation between the k values obtained at different incubation periods: between week 2 and week 5 ($p<0.001$), week 6 and week 2 ($p<0.001$) (Appendix 10). Generally litter incubated at high temperature recorded high k values. Decomposition constant values decreased as the decomposition progressed i.e. week 16 generally recorded low k values than week 2.

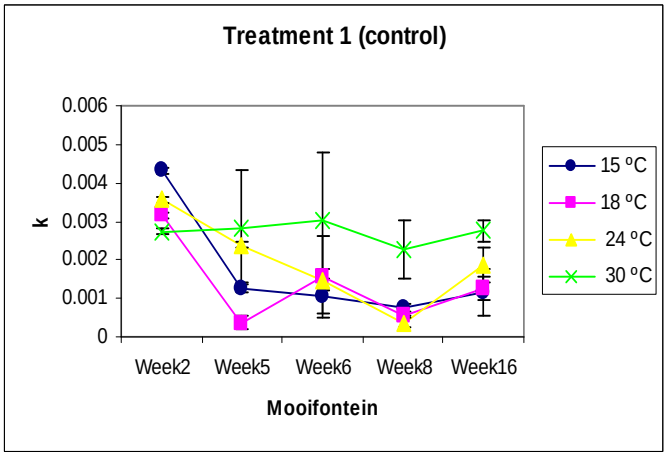
Figure 4.8 Decomposition constants of litter from three unfertilized and fertilized sites incubated for up to 16 weeks at four temperatures. Values are mean ±SE

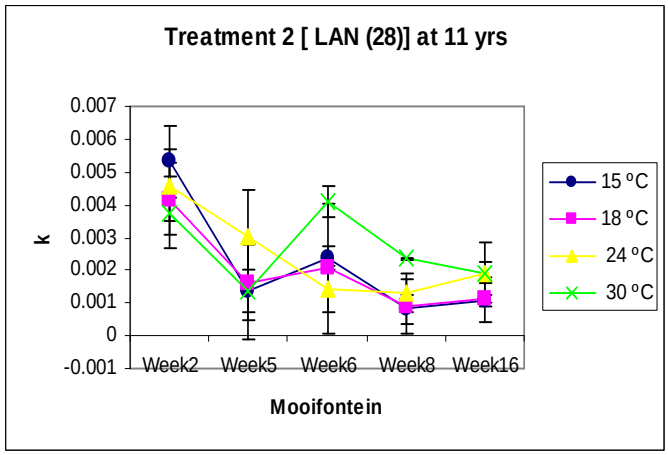


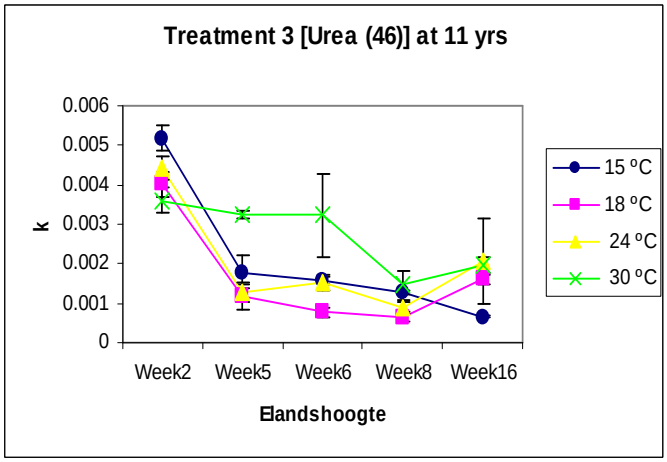


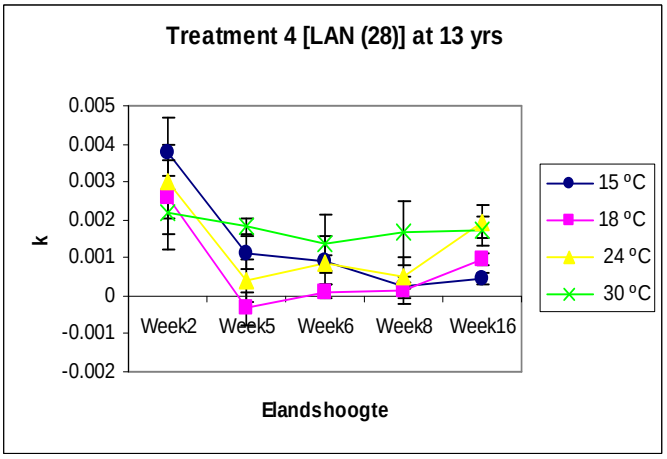


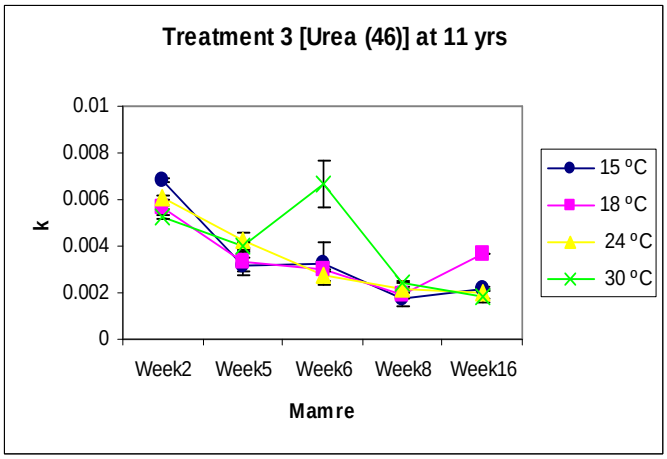


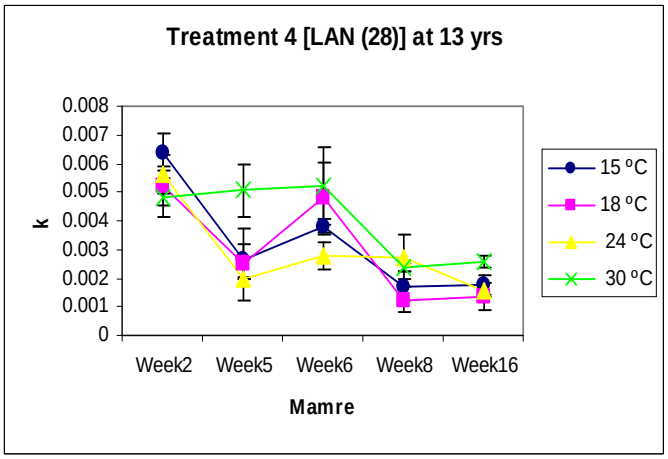


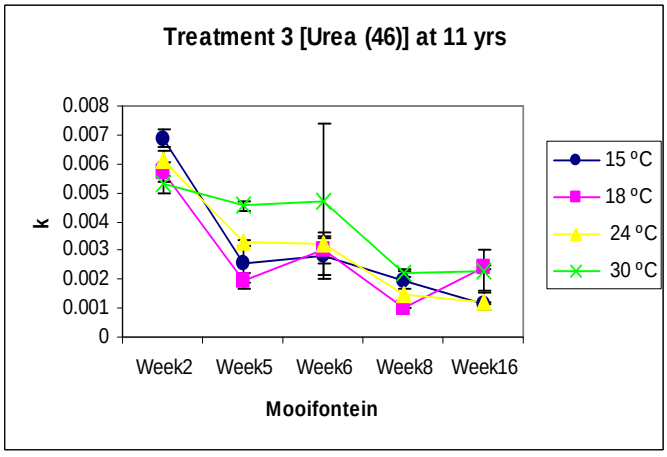


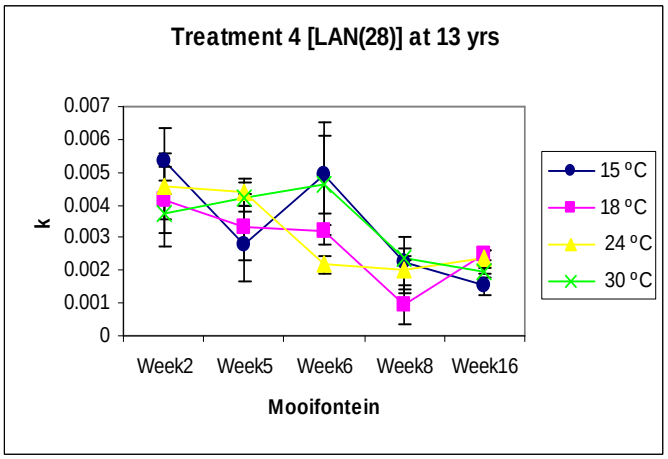

Treatment 3 [Urea (46)] at 11 yrs
0.006
0.005
0.004
0.003
0.002
0.001
0
k
Week2
Week5
Week6
Week8
Week16
Elandshoogte
15 °C
18 °C
24 °C
30 °C

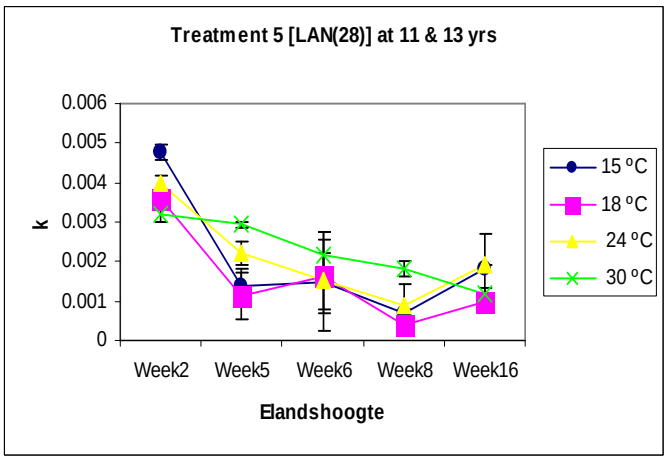

Treatment 4 [LAN (28)] at 13 yrs
0.005
0.004
0.003
0.002
0.001
0
-0.001
-0.002
k
Week2
Week5
Week6
Week8
Week16
Elandshoogte
15 °C
18 °C
24 °C
30 °C

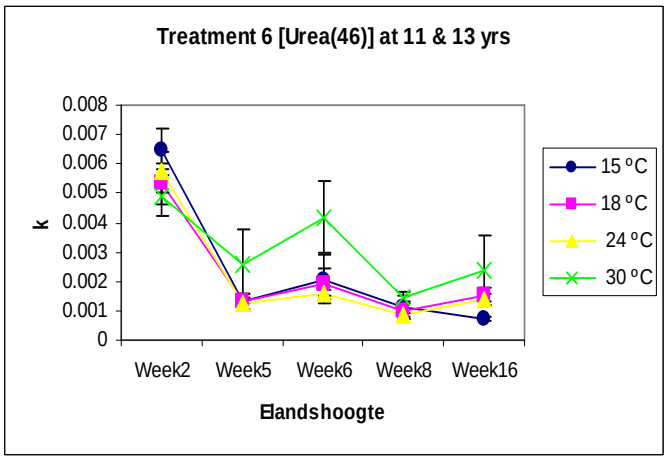

Treatment 3 [Urea (46)] at 11 yrs
0.01
0.008
0.006
0.004
0.002
0
k
Week2
Week5
Week6
Week8
Week16
Mamre
15 °C
18 °C
24 °C
30 °C

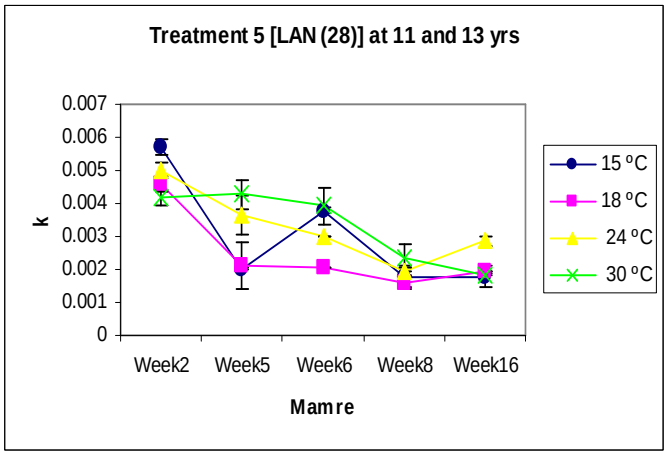

Treatment 4 [LAN (28)] at 13 yrs
0.008
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0
k
Week2
Week5
Week6
Week8
Week16
Mamre
15 °C
18 °C
24 °C
30 °C

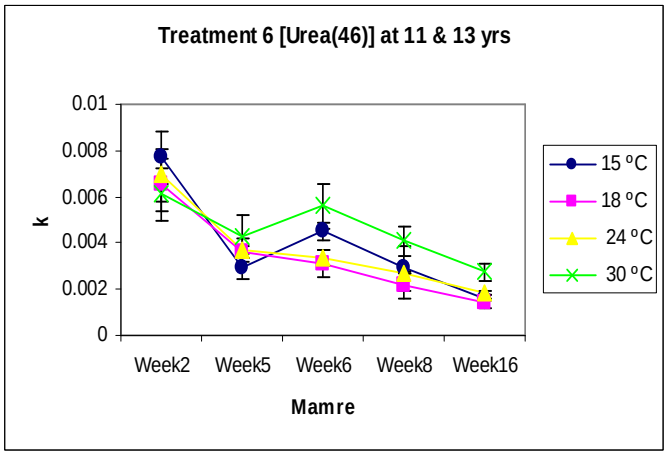

Treatment 3 [Urea (46)] at 11 yrs
0.008
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0
k
Week2
Week5
Week6
Week8
Week16
Mooifontein
15 °C
18 °C
24 °C
30 °C

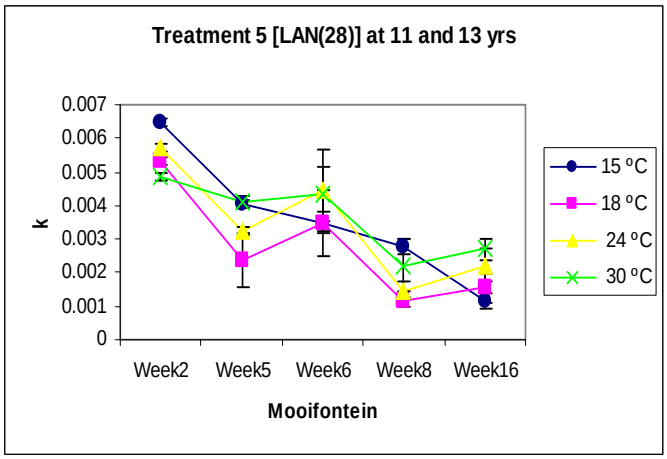

Treatment 4 [LAN(28)] at 13 yrs
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0
k
Week2
Week5
Week6
Week8
Week16
Mooifontein
15 °C
18 °C
24 °C
30 °C

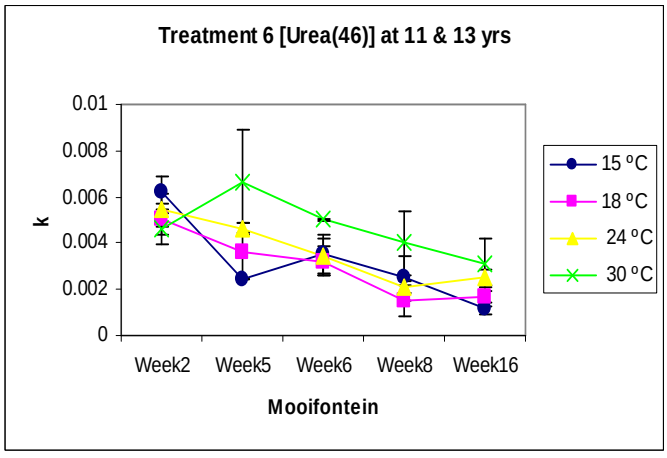

Treatment 5 [LAN(28)] at 11 & 13 yrs
0.006
0.005
0.004
0.003
0.002
0.001
0
k
Week2
Week5
Week6
Week8
Week16
Elandshoogte
15 °C
18 °C
24 °C
30 °C


Treatment 6 [Urea(46)] at 11 & 13 yrs
0.008
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0
k
Week2
Week5
Week6
Week8
Week16
Elandshoogte
15 °C
18 °C
24 °C
30 °C


Treatment 5 [LAN (28)] at 11 and 13 yrs
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0
k
Week2
Week5
Week6
Week8
Week16
Mamre
15 °C
18 °C
24 °C
30 °C


Treatment 6 [Urea(46)] at 11 & 13 yrs
0.01
0.008
0.006
0.004
0.002
0
k
Week2
Week5
Week6
Week8
Week16
Mamre
15 °C
18 °C
24 °C
30 °C


Treatment 5 [LAN(28)] at 11 and 13 yrs
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0
k
Week2
Week5
Week6
Week8
Week16
Mooifontein
15 °C
18 °C
24 °C
30 °C


Treatment 6 [Urea(46)] at 11 & 13 yrs
0.01
0.008
0.006
0.004
0.002
0
k
Week2
Week5
Week6
Week8
Week16
Mooifontein
15 °C
18 °C
24 °C
30 °C

Chapter 5- Discussion

5.1 Litter chemistry and nutrient dynamics

The litter N concentrations recorded in this study can be compared with other studies on the same species in the summer rainfall regions of South Africa. The N values recorded for needle litter (mean=1.42 %) were considerably higher than that found by Bird (2001) in the Ermelo district of the Mpumalanga Highveld. Morris (1993) working in *P. patula* plantations in Swaziland found mean litter N values of 1.25%. Lemma *et al.* (2007) recorded a value of 0.56% using freshly fallen needle litter collected from *P. patula* in the Southwestern highlands of Ethiopia. Nitrogen concentrations of *P. patula* in this study varied greatly when compared with values of other pine species. Maggs (1988) in *P. elliottii* stands found N concentrations of 0.33% and 0.59% for layers L and F2 respectively. Sharma and Pande (1989) reported a value of 0.8% for pine plantations in India.

The differences in nutrient concentrations between those from the current study and those reported by various authors in the literature can be explained by the different species that were under investigation, site differences, the degree to which the litter had been exposed to decomposition, seasonal variation and age of the trees. Phenologically different species may resorb N and P before the abscission of leaves to redeploy in developing tissue in order to minimize nutrient losses (Killingbeck, 1996) more so when plants experience nutrient deficiency (Aerts, 1996). For example Olbrich (1994) cited in Bird (2001) found internal translocation in *P. patula* occurring at two distinct periods: spring to early summer and late summer to winter. Resorption and internal translocation will therefore reduce the foliar nutrient concentration and thus reduce the level of nutrients in the litter. The age of the stand from which the litter is collected influences the nutrient content of the litter. Morrison (1974) cited in Louw and Scholes (2003) observed the influence of tree age on litter N content with the highest levels occurring at the age of approximately 30 years which possibly explains the difference in litter N content in the current study (1.42% N content from a 18 year old stand) and the results of Bird (2001) of 1.01% N

content from a *P. patula* stand of 16 year. Season is another factor that may affect the litter nutrient content. Louw and Scholes (2003) report mean values of 1.24% foliar N during winter while, the value of 1.79% was reported during the summer period. The length of time that litter has been exposed to decomposers will influence the quantity of nutrients found in leaf litter, as illustrated by Lemma *et al.,* (2007). They found that newly shed leaf litter had N concentrations of 0.56% while the litter from the Oe layer of the forest floor (decomposed) had N concentration of 1.6%. Similarly Maggs (1988) found that in *P. eliiottii* forest floor the L (Litter) and F1 (first fermentation) layers were found to have 0.33 and 0.44% N respectively. The increase in N (%) during decomposition can be attributed to net loss of carbon in the form of CO_2 combined with microbial immobilization (Maggs 1988, Ribeiro *et al.,* 2002).

The litter N concentration varied with the time and frequency of fertilizer application. Litter collected from plots fertilized at 13 years recorded higher N concentrations than those of 11 years, implying that the effect of fertilization on litter N concentration diminished as the time progressed. The litter collected from plots subjected to fertilization at 11 and 13 years recorded higher concentrations of N than those fertilized at only 11 or 13 years, this trend can possibly be explained by the residual effect of fertilization at 11 years. Crous *et al.* (2007) found a significant effect of residual fertilizer on litter P levels when additional fertilizers were added to *P. patula* stands in Usutu, Swaziland. Similar results were found in *P. resinosa* stands which were fertilized with N for 9 years at Harvard forest, where the N concentration of litter continuously increased (Magill *et al*., 2000).

The relationship between litter chemistry and decomposition was investigated by comparing the chemistry of fresh leaf litter with the litter that was incubated for 16 weeks. The effect of temperature, fertilization and site on nutrient concentration was also investigated.

Decomposed litter showed higher N concentrations and lower C:N ratios compared with fresh leaf litter, consistent with the pattern documented in many other litterbag and laboratory studies (Cuevas and Medina 1988, Louw and Scholes 2002, Heim and Frey, 2004 Lemma *et al.,* 2007). This pattern was attributed to net losses of C (or mass losses)

combined with microbial immobilization (Maggs 1988, Ribeiro *et al.,* 2002) and the slow biological breakdown of nitrogen-substituted lignin (Melillo *et al*., 1982; Couteaux *et al*., 1995). Temperature affected the N accumulation of the litter. Higher temperatures resulted in more accumulation of N. Hobbie (1996) investigating the difference in N release of the litter incubated at 4 ºC and 10 ºC found initial N loss followed by N gain, followed by another period of N loss at 10 ºC, however the 4 ºC treatment litter did not exhibit a final period of N loss. However, Shaw and Harte (2001) found no effect of warming on nitrogen release. Site also significantly affected N accumulation of the litter. This difference can possibly be explained by the fact that the initial amount of N concentration of the freshly fallen litter was different and therefore the effect is reflected on the decomposed litter. For example in a study by Ribeiro *et al.* (2002) the litter with an initial higher concentration of N and P released higher proportions of these elements which is consistent with the current study where there was a positive correlation between the initial N concentration of the litter and the decomposed litter. The result of this study therefore supports the hypothesis that future climate warming will directly affect N and C cycling of the forests.

5.2 Rate of CO_2 production from the litter

5.2.1 Temperature, fertilizer, time and site effects

Increasing atmospheric temperature may alter C cycling in forest ecosystems through changes in decomposition, and possibly act as a source of CO_2 to the atmosphere as positive feedback. This study mainly evaluated the effect of temperature on the rates of CO_2 production from litter at an early stage of decomposition. Temperature had a significant effect on rates of CO_2 production with higher temperatures resulting in higher production of CO_2 from the litter. Other studies (Hobbie, 1996; Kim *et al.,* 2005) have found CO_2 emissions from litter or soil to be strongly stimulated by temperature. Under a warming climate with possible increases in temperature of 1-3 ºC in the Southern African region, the amount of CO_2 emissions from the forest floor is expected to increase. Seasonal variation will be important in determining the pattern of CO_2 emission from the litter as there was marked difference in the amount of CO_2 produced at 30 ºC and 24 ºC when compared with litter incubated at 15 ºC and 18 ºC. At 30 ºC the CO_2 emission declined even though mass loss continued to increase. The amount of CO_2 emission was small (mg) relative to mass loss (g) and therefore continued increase in mass loss at 30 ºC even as CO_2 emission decreased can possibly be attributed to other metabolic changes taking place in the litter. Seasonal differences may also be important as during the drier, winter months or under drought conditions the composition of the microbial community may change with fungi becoming more dominant which may affect the amount of CO_2 production from the forest floor.

The amount of CO_2 produced was also affected by fertilization and duration of incubation. As time progressed, the amount of CO_2 produced increased in magnitude. This is in agreement with the results of Witkamp (1966a) who found positive correlations between the amount of CO_2 produced and time. Although temperature accounted for much of the variability of CO_2 produced, the significant two and three way interactions between temperature, fertilization, site and time indicated that the effect of temperature was not consistent across fertilization or sites. The effects of fertilization on

decomposition rate of the litter and soil organic matter have been controversial. For example a study by Bradford *et al*. (2008) found that P amendment stimulated decomposition rates while N fertilization suppressed decomposition of organic matter. In the context of feedback of CO_2 from the litter and soil as a result of global warming, the option of fertilizer application to forest plantation should be based on whether the amount on CO_2 emissions from the forest as a result of fertilization outweigh the amount of C that can be sequestered by the forest plantation.

There was no significant effect of site on the amount of CO_2 produced from the litter collected from various study sites, a possible explanation for this is that the amounts of C in the litter collected from the different sites were more or less the same and therefore the amount of carbon respired in the form of CO_2 may reflect the carbon content of the litter collected from different study sites. Other studies have found significant site effects on factors affecting litter decomposition for example studies conducted in India by Kshattriya *et al*. (1992) found that fungal and bacterial counts and invertase, amylase and cellulase enzyme activities were greatest on litter in low altitude sites when compared with high altitude sites. Decomposition at low altitude sites was found to be higher than high altitude sites in Mpumalanga region of South Africa (Dames, 1996) implying that places with different altitudes will have different rates of CO_2 emissions due to influence of altitude on temperature and microbial population and activity. In the current study although site effect was not significant the amount of CO_2 produced based on altitude of the site revealed that the site at lower altitude (Mooifontein) recorded higher amounts of CO_2 emissions than Elandshoogte and Mamre.

5.3 Mass loss

5.3.1 Temperature effect

For terrestrial ecosystems, climate variables (temperature and moisture) have been found to be a rate regulating factor of litter decomposition (Aerts 1997). Temperature is often the primary factor determining rates of litter decomposition (Meentemeyer 1978). In the current study there were significant temperature effects on litter mass loss which are consistent with results of previous studies (Meentemeyer 1978, Hornsby *et al.,* 1995,

Hobbie 1996) which found that mass loss and decomposition rates increased with an increase in temperature. A possible explanation for this pattern is the effect of increased temperature on microbial activity. Witkamp (1966a) found that increased temperature increased the population of microorganisms and accelerated the amount of CO_2 produced from the litter. Witkamp (1966a) and Aerts and De Caluwe (1997) found significant correlations between mass loss and respiration rates of the litter which is consistent with the current study where the correlation between cumulative mass loss and cumulative CO_2 emission was found to be positive (r=0.212) and significant when tested at 0.1 significant level (p=0.0741). Fierer *et al.,* (2005) attributed the majority of the C respired over relatively short incubation periods to loss of the soluble C fraction. The C loss in the form of CO_2 from the litter possibly explains the mass loss recorded during the study.

Mass loss of the litter and temperature exhibited linear relationship where litter incubated at 30 ºC recorded the highest mass loss and 15 ºC the lowest. Temperatures (15º, 18º, 24º and 30 ºC) were chosen bearing in mind that the study area currently experiences a mean annual temperature of 15 ºC and a possible temperature increase for the area of 3 ºC (Choice of 18 ºC). Temperatures 24 ºC and 30 ºC were included in the experiment to investigate the response of decomposition at higher temperatures.

5.3.2 Litter quality effect

Several studies have indicated that the chemical and biochemical quality of litter affects mass loss during decomposition (Berg 1986, Vitousek *et al.,* 1994). In many studies N concentration of the litter was strongly correlated with litter mass loss (Witkamp 1966a, Taylor *et al.,* 1989). Mass loss in the current study was found to be only very weakly correlate with N (%) of the litter. The quantity of N in the litter has been found to affect the activity of microorganisms. Litter with higher N concentrations is readily decomposed by microorganisms (Swift *et al.,* 1979) and this possibly explains why litter with higher N lost more mass than those with lower N concentrations. The effect of N concentration on litter mass loss showed some variation as decomposition progressed.

During the initial stage (2-8 weeks) the correlation between N % and mass loss was stronger than at the later decomposition phase (16 weeks). This is in agreement with Cotrufo *et al.* (1995) who reported that litter mass loss was positively correlated with N concentration during the early phase of decomposition but the influence diminished at the later stage of decomposition. A possible explanation for the diminishing role of N in explaining mass loss of the litter as decomposition progressed is that N has been found to limit decomposition at high concentrations. Nitrogen at high concentrations reacts with lignin and polyphenols at a late stages of decomposition creating more recalcitrant compounds (Ågren *et al*., 2001), suppresses activity of lignin-degrading fungi (Berg, 2000) and decreases the amount of fungi and bacteria (Fang *et al*., 2007). Other studies have shown that after an initial period during which various compounds are lost, the rate of decomposition decreases. This is caused by the accumulation of condensed or polymerized polyphenols which may originate from the litter or be synthesized by microorganisms (Singh and Gupta 1977, Dames, 1996).

5.3.3 Fertilization effect

Previous studies that have investigated the effects of fertilization on the rate of litter decomposition have been controversial. In this study fertilization had significant effects on mass loss of the litter in some cases. Litter from fertilized plots decomposed faster when compared with the control. This is consistent with some other studies (Hunt *et al.*, 1988, Ostertag and Hobbie 1999, Li *et al.*, 2006). The effects of fertilization on decomposition rate can possibly be explained by the effects of fertilizer on litter quality. Fertilization increased the N concentration of the litter which in turn increased the amount of mass loss of the litter collected from fertilized plots. However, some fertilized plots did not show any differences when compared with the control. The inconsistent result may be due to the significant interaction between the site and fertilization treatment that was evident.

5.3.4 Site effect

Needle litter collected from different sites showed different magnitudes of mass loss. The difference in mass loss is possibly explained by differences in the amount of N contained in the litter collected from different sites. The sites (Mooifontein and Mamre) with high litter N concentration lost more mass than the site Elandshoogte with low N concentration. Initially litter that contained high amounts of N lost more mass than litter that contained low amounts of N. litter collected from the sites on shale recorded more mass loss than site the on andesite. The sites had different soil textures where litter collected from clay loam soils recorded more mass loss than Elandshoogte with a clay soil texture. Soil texture has been found to influence the rate of N mineralization and soil N concentration. Nitrogen mineralization is generally more rapid in coarse than fine texture soils (Ladd *et al*., 1992; McLauchlan, 2006). The rate of N mineralization possibly affected the nitrogen concentration of the litter and the effect reflected on mass loss of the litter. In this study the effect of site on mass loss was only related to litter chemistry and temperature of incubation but under field conditions the result may differ due to different factors affecting decomposition of the litter including both biotic and abiotic factors. Abiotic factors include the litter's physical structure, temperature, moisture, relative humidity and pH, while biotic factors include litter quality, microbial activity and the composition of the soil microorganisms and soil fauna (Dickinson and Pugh, 1974; Swift *et al*., 1979). Furthermore, the values of the mass loss in the laboratory may be underestimated because macrofauna like earthworms were not involved in the decomposition of the litter. Soil macrofauna are known to contribute significantly to the breakdown of leaf and needle litter by fragmentation which favours the colonization of bacteria and fungi by increasing the available surfaces and hence may accelerate decomposition (Soma and Saito, 1983; Kheirallah 1990). The differences in the amount of mass loss recorded in different sites reflects the influence of site characteristics on decomposition rate of the litter which could have not been detected if the litter was collected from only one site.

5.3.5 Time effect

Generally the amount of mass loss increased as decomposition progressed. Highest mass loss was recorded at week 16. This is possibly explained by loss of labile C fractions and other soluble fractions which are normally lost during the early decomposition phase (White et al 1988; Fierer *et al.,* 2005). As the time progresses there are usually the formation and accumulation of humic materials which normally have a very slow rate of decomposition (Swift *et al.,* 1979). Li *et al.* (2006) for example using *Dacryodes excelsa* leaf litter found that mass loss rate declined with the incubation time. Leaf litter decayed fast during the first 3 months and after 8 months of incubation the leaf mass loss slowed down and approached a constant value through the next 4 months of the study period. In the Mpumalanga region of South Africa rapid mass loss of *P. patula* litter occurred during the first six weeks. Mass loss of the current study is consistent with previous study in Mpumalanga (Dames, 1996).

5.4 Decomposition rate constant (k)

5.4.1 Temperature, fertilizer, time and site effects

Temperature, fertilizer and site effects on decomposition rate mirrored mass loss data. The only difference with regard to mass loss is the time effect where decomposition rate values decreased as the time of decomposition progressed. The lower k values were recorded at week 8 and week 16, the reason being the duration of incubation being longer than weeks 2, 5 and 6.

Chapter 6- Conclusions

This study revealed that temperature affects mass loss, CO_2 emission and N release from decomposing needle litter of *P. patula*. Decomposition rate of the litter was accelerated by an increase of 3 ºC in temperature implying that future warming will directly affect cycling of C and N in *P. patula* litter and soil of South Africa. The study confirms that there is a strong potential positive feedback inherent in the massive amounts of carbon that are currently tied up in the litter on the forest floor. The danger is that this could be released by global warming and greatly add to the CO_2 already in the atmosphere. The decomposition rate of the litter was more marked at higher temperatures (24 and 30 ºC) implying that seasonal variation will play a major role in the flux of CO_2 and mass loss of the litter.

The release of nitrogen from the litter increased with temperature therefore global warming will have an effect on nitrogen availability to the forest. Application of fertilizer differentially affected the decomposition rate of the litter as some fertilizer treatments were significantly different from the control while others were not. There is no simple explanation as to why this is the case.

The nitrogen concentrations in the needles of fertilized plots were significantly higher than the control. The amount and type of fertilizer applied also had minor effects on litter N concentration. Litter collected from *P. patula* plots that were fertilized with urea recorded higher N concentrations than those that underwent limestone ammonium nitrate fertilization. The plots that were subjected to fertilization at 11 and 13 years recorded higher N concentration than those fertilized at only 11 or 13 years indicating that there was a residual effect of the fertilizer earlier applied. Therefore, the amount and type of fertilizer application will affect the litter N concentration and decomposition rate.

The litter quality was not a strong predictor of litter decomposition rates implying that temperature will be the major factor influencing the decomposition rate of *P. patula* needle litter. Mass loss, CO_2 emission and nitrogen release of the *P. patula* needle litter appear to be strongly influenced by the site differences. Study by Schutz's (1990) cited in Dames (1996) found that site factors correlated with litter decomposition. Litter collected

from sites on shale with a clay loam soil texture had higher decomposition rates than those collected from the site on andesite geology and clay soil texture implying that decomposition rate of the litter will be site specific. Additionally, the decomposition of the litter is determined by the interplay of site, temperature and duration of incubation, therefore there is a need to develop models to assess the decomposition of the litter based on factor interactions.

Chapter 7 References

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Chapter 8 Appendices

Appendix 1 Initial litter chemistry parameters as a function of site and N fertilization (Two-way ANOVA)

Initial litter chemistry	Sites df(2)	N fertilization df(5)	Interaction df(10)
N concentration	0.0017*	0.4558	0.6931
C concentration	0.3163	0.5597	0.7302
C:N ratio	0.0008**	0.4018	0.9848

p-values are represented with their level of significance *p<0.01 **p<0.001

Appendix 2 Litter N (%) increase after 16 weeks of incubation as function of temperature (Temp), site and fertilization (Fert) (Three-way ANOVA).

Source of variation	df	F	p
Temp	3	4.8503	0.0082
Fert	5	2.5675	0.0291
Site	2	7.099	0.0032
Temp:Fert	15	1.4273	0.1582
Temp:Site	6	0.723	0.6324
Fert:Site	10	2.8041	0.3542
Temp:Fert:Site	30	1.0651	0.4024

Appendix 3 Litter C:N ratio after 16 weeks of incubation as a function of temperature (Temp), site and fertilization (Fert) (Three-way ANOVA).

Source of variation	df	F	p
Temp	3	4.8503	0.0325
Fert	5	2.5675	0.0669
Site	2	7.099	<0.01
Temp:Fert	15	1.4273	0.1303
Temp:Site	6	0.723	0.8189
Fert:Site	10	2.8041	0.4873
Temp:Fert:Site	30	1.0651	0.2686

Appendix 4 The effect of the temperature (Temp), site, fertilization (Fert) and time of incubation on CO_2 production from the litter (ANOVA results)

Source of variation	df	F	p
Temp	3	192.54	<0.001
Site	2	0.3422	0.7104
Fert	5	4.2935	<0.001
Time	3	452.285	<0.001
Temp:Fert	15	2.6993	<0.001
Temp:Site	6	3.4022	0.0029
Fert:Site	10	2.3102	0.0126
Temp:Time	9	96.67	<0.001
Fert:Time	15	2.5487	0.0014
Site:Time	6	0.3824	0.89
Temp:Fert:Site	30	2.5148	<0.001
Temp:Fert:Time	45	1.7637	0.0032
Temp:Site:Time	18	2.4676	<0.001
Fert:Site:Time	30	1.8183	0.007
Temp:Fert:Site:Time	90	2.6713	<0.001

Appendix 5 The effects of temperature, site and fertilization on CO_2 production at four incubation periods (ANOVA results)

Period	Source of variation	df	F	p
Week 2	Temp	3	14.2017	<0.001
	Site	2	1.725	0.1854
	Fert	5	1.0177	0.4137
	Site:Fert	10	2.6251	0.0088
	Site:Temp	6	13.6342	<0.001
	Fert:Temp	15	3.1779	0.0005
	Site:Fert:Temp	30	2.6714	0.0004
Week 6	Temp	3	208.09	<0.001
	Site	2	0.1111	0.895
	Fert	5	13.032	<0.001
	Site:Fert	10	4.943	<0.001
	Site:Temp	6	9.6373	<0.001
	Fert:Temp	15	8.9881	<0.001
	Site:Fert:Temp	30	9.6744	<0.001
Week 8	Temp	3	94.3001	<0.001
	Site	2	0.8415	0.4352
	Fert	5	0.9629	0.4464
	Site:Fert	10	0.8423	0.59
	Site:Temp	6	1.0418	0.4057
	Fert:Temp	15	0.6992	0.77
	Site:Fert:Temp	30	1.665	0.0406
Week 16	Temp	3	111.44	<0.001
	Site	2	0.1144	0.8921
	Fert	5	1.0317	0.4057
	Site:Fert	10	1.7746	0.0809
	Site:Temp	6	1.6573	0.144
	Fert:Temp	15	0.7116	0.7649
	Site:Fert:Temp	30	1.0968	0.3657

Appendix 6 The effect of the temperature (Temp), site and fertilization (Fert) on cumulative CO_2 production from the litter (ANOVA results)

Source of variation	df	F	p
Temp	3	74.19	<0.001
Site	2	0.1344	0.8745
Fert	5	1.6632	0.1571

Appendix 7 The effects of temperature (Temp), site, fertilization (Fert) and time on mass loss of the needle litter (ANOVA results).

Source of variation	df	F	p
Temp	3	43.87	<0.001
Fert	5	10.5	<0.001
Time	4	178	<0.001
Site	2	96.22	<0.001
Temp:Fert	15	1.249	0.2326
Temp:Time	12	10.28	<0.001
Fert:Time	20	0.466	0.9772
Temp:Site	6	1.039	0.3993
Fert:Site	10	9.307	<0.001
Time:Site	8	3.604	0.0004
Temp:Fert:Time	60	1.18	0.184
Temp:Fert:Site	30	0.737	0.8437
Temp:Time:Site	24	0.768	0.7773
Fert:Time:Site	40	1.035	0.4167
Temp:Fert:Time:Site	120	0.925	0.69

Appendix 8 The effects of temperature, site and fertilization on mass loss of the litter at four incubation periods (ANOVA results)

Period	Source of variation	df	F	p
Week 2	Temp	3	13.50	<0.001
	Site	2	14.94	<0.001
	Fert	5	10.24	<0.001
	Site:Fert	10	4.034	<0.001
	Site:Temp	6	<0.001	1.0000
	Fert:Temp	15	<0.001	1.0000
	Site:Fert:Temp	30	<0.001	1.0000
Week 5	Temp	3	26.62	<0.001
	Site	2	39.39	<0.001
	Fert	5	3.216	0.0112
	Site:Fert	10	5.002	<0.001
	Site:Temp	6	0.333	<0.9174
	Fert:Temp	15	0.731	0.7446
	Site:Fert:Temp	30	0.960	0.5363
Week 6	Temp	3	15.36	<0.001
	Site	2	30.76	<0.001
	Fert	5	2.645	0.0298
	Site:Fert	10	3.695	<0.001
	Site:Temp	6	0.629	0.7064
	Fert:Temp	15	0.910	0.5563
	Site:Fert:Temp	30	0.806	0.7402
Week 8	Temp	3	10.08	<0.001
	Site	2	21.48	<0.001
	Fert	5	2.888	0.0200
	Site:Fert	10	2.444	0.0141
	Site:Temp	6	0.787	0.5827
	Fert:Temp	15	0.454	0.9556
	Site:Fert:Temp	30	0.516	0.9767
Week 16	Temp	3	19.04	<0.001
	Site	2	11.47	<0.001
	Fert	5	1.295	0.0197
	Site:Fert	10	1.202	0.0141
	Site:Temp	6	1.184	0.5827
	Fert:Temp	15	2.022	0.9556
	Site:Fert:Temp	30	1.226	0.9767

Appendix 9 The effects of temperature (Temp), site and fertilization (Fert) on cumulative mass loss of the needle litter (ANOVA results).

Source of variation	df	F	p
Temp	3	43.87	<0.001
Fert	5	10.5	0.0017
Site	2	96.22	<0.001

Appendix 10 The effects of temperature (Temp), site, fertilization (Fert) and time on decomposition rate of the needle litter (ANOVA results).

Source of variation	df	F	p
Temp	3	29.562	<0.001
Fert	5	15.353	<0.001
Time	4	103.402	<0.001
Site	2	284.353	<0.001
Temp:Fert	15	0.776	0.705
Temp:Time	12	0.366	0.900
Fert:Time	20	12.405	0.059
Temp:Site	6	14.049	0.900
Fert:Site	10	1.562	<0.001
Time:Site	8	4.291	<0.001
Temp:Fert:Time	60	0.580	0.957
Temp:Fert:Site	30	0.695	0.964
Temp:Time:Site	24	0.563	0.954
Fert:Time:Site	40	1.643	0.010
Temp:Fert:Time:Site	120	0.706	0.987