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By
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הגורמים המשפעים על זמינות חנקן ביער הגדל באזור יבש

Factors controlling availability and dynamics of nitrogen in a semi-arid forest ecosystem

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מוגש למועצה המדעית של
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This work has been conducted under the supervision of

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This work is dedicated to my family, my wife Ira, my mother and father, and my brothers, who always support and understand me.

Declaration

All group members associated with the Yatir Forest flux tower research site have been involved in the maintenance and operation of the site. I used the existing datasets of soil temperature and water, and flux data from Yatir Forest project, but all interpretations and analyses are my own. All the work and results presented in this thesis are my own.

Ilya Gelfand

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Abstract

Land use and afforestation are important factors in the contemporary carbon cycle and influence the airborne fraction of anthropogenic CO_2 emissions. Here we studied ecosystem-scale changes in nitrogen dynamics associated with afforestation in a semi-arid shrubland in southern Israel. This was motivated by the previous observations that the 40 years old pine afforestation system resulted in 2.5 fold increase in carbon stock, and mean annual NEE (last 6 years) of 2.6 tC ha^{-1}, similar to the global mean in the FluxNet monitoring system. The results of the present study show that there were only minor changes in ecosystem nitrogen stocks. However, there was almost 5 fold increase in the nitrogen use efficiency (NUE), compared to the surrounding shrubland (406.4 vs. 86.9, gr/gr based on ANPP, N stock and N turnover rate). Leaf C/N ratios increased from 22 in the shrubland to 53 in the forest, and litter C/N increased from 38 to 146, consistent with the increase in NUE and indicating efficient N re-allocation in the forest. Mineralization and decomposition rates were slowed down by ~50% (with large variability), and ~20% respectively. These reductions in N recycling rates were accompanied with ~65% decrease in annual nitric oxide emission in the forest ecosystem (with large seasonal cycle), and accumulation of soil NO_2^- in the beginning of the wet period. The nitrite accumulation indicated differential recovery from the long dry season of microbial populations that oxidize ammonium to nitrate, which improved synchronization of nitrate supply to peak plant activities. The range of adjustments observed in the ecosystem N cycle help explain the significant carbon sequestration and biomass storage in this semi-arid forest, without significant changes in N inputs. This, in turn, indicated that long-term warming and drying trends predicted for the entire Mediterranean, and other regions, may have milder effects on ecosystem productivity and carbon sequestration than predicted from short term drought or heat episodes in temperate regions.

תקציר

כיום ניתן לאמר בוודאות (IPCC 2007) כי פעילות האדם גורמת לשינויים במחזור הפחמן הגלובלי וכתוצאה מכך גם לחלק משינויי האקלים הנצפים בשנים האחרונות. תחזיות לעשורים הקרובים צופות התחממות ניכרת כתוצאה משינויים אלו. מעגל הפחמן, ובפרט ריכוזי הפחמן הדו-חמצני באטמוספרה, מושפעים רבות מתהליכים הגורמים לשינויים בשימושי הקרקע ובייעור. כמו כן, קיים קשר ישיר והדוק בין מעגלי הפחמן והחנקן בטבע.

בעבודה זו מוצגות תוצאות מחקר שבדק את השינוים שחלו עקב תהליכי ייעור במערכת שיחנית (shrubland) בנגב הצפוני. המחקר בוצע בשתי רמות: רמת המערכת האקולוגית ורמת התהליכים הספציפיים בקרקע. ארבעים שנה לאחר שתילת העצים, נמצא שינוי משמעותי במאגרי הפחמן במערכת היער לעומת המערכת השיחנית (פי 2.5 יותר פחמן נמצא ביער). כמו כן, נמצא כי תהליכי הייצור הראשוני במערכת הייער גבוהים יחסית - בשש השנים האחרונות מערכת היער קלטה כל שנה בממוצע 2.6 $tC\ ha^{-1}$, שטף הדומה בגודלו לממוצע של מערכות היער בחצי כדור הארץ הצפוני. לעומת זאת, לא נמצאו שינוים או הבדלים מהותיים במאגרי החנקן בין מערכת הייער והמערכת השיחנית. חוסר הבדל מהותי במאגרי החנקן בין שתי המערכות התבטא בהגדלת ה-NUE (יעילות שימוש בחנקן, Nitrogen Use Efficiency), שנמצאה גדולה פי 5 ביער לעומת המערכת השיחנית (406.4 ו-86.9 גר $גר^{-1}$, מחושב על בסיס הייצור הראשוני מעל הקרקע, מאגרי החנקן וקצב המחזור). יחס הפחמן לחנקן בעלווה השתנה עקב הייעור מ-22 ל-53 בעלים חיים ומ-38 ל-146 בנשר, יחס שנמצא עיקבי לעליה ב- NUE ומציין שימוש חוזר יעיל בחנקן לפני נשירת העלים.

לא נמצא שוני משמעותי בכמות החנקן הנכנס למערכות מהאטמוספרה על ידי אבק או גשם. הגשם מכניס ליער בקירוב 1.8 ק"ג חנקן יותר מאשר למערכת השיחית. קצבי פירוק הנשר והפיכת החומר האורגאני לחנקן מינראלי (Mineralization) הושפעו על ידי הייעור והראו קצבים הנמוכים בכ-20% וכ-50% בהתאמה בתוך היער (עם שונות גבוהה). יחד עם ההאטה בקצבי מחזור החנקן במערכת יער, גם פליטת תחמוצת החנקן (NO) מהקרקע קטנה בכ-65%. יתרה מכך, לאחר ההרטבה הראשונית של הקרקע נמדדו ריכוזים גבוהים יחסית של ניטריט (NO_2^-) בקרקע, תצפית המצביעה על קצבי התאוששות שונים בין חיידקים המחמצנים אמוניה וניטריט לאחר תקופת היובש. ירידה בפליטת תחמוצת החנקן והצטברות הניטריט מצביעים על התאמה בין הצורך בחנקן וזמינות החנקן במערכת יער.

השינוים במעגל החנקן שנמצאו במחקר זה מאפשרים להסביר את קצבי קיבוע ואחסון הפחמן הגבוהים שנמדדו במערכת המיוערת. אי לכך, יתכן ותהליכי ההתחממות וההתייבשות הצפוים להתרחש באיזור הים התיכון ישפיעו פחות מהמצופה על היצור הראשוני, וזאת בניגוד להשערות קודמות שהתקבלו על בסיס תצפיות קצרות מועד כגון מקרי בצורת.

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Chapter 1

1 Introduction

1.1 The global nitrogen cycle

The main reservoir of nitrogen (N) on the Earth is the atmosphere, which contain $4x10^9$ Tg N (1 Tg = 10^{12} g; Table 1.1; Reeburgh, (1997)). However, atmospheric N exists in stable, di-nitrogen form (N_2) with a strong triple bond between the two N atoms and therefore is not readily available for biological activity. The only sources for biologically available forms of N are biological N fixation (production of ammonium) and, to a lesser extent, lightening (production of N oxides). These two processes account for fixation of 90 - 130 Tg N y^{-1} in the terrestrial and 40 - 200 Tg N y^{-1} in the marine ecosystems (Galloway, 1998). In marine environments, fixed N has a fast turnover rate (marine biomass; Table 1.1) and little of this N is transported to the terrestrial biomes. In terrestrial ecosystems, the main reservoir of N is soils, where N often exists as part of complex organic molecules and is not readily available for vegetation (Vitousek and Howarth, 1991). The organic N in the soils is, however, subject to mineralization to inorganic forms by microorganisms and it is through the mineralization process that soil N becomes available for plant uptake. Therefore, mineralization is one of the main processes controlling the N cycle in terrestrial ecosystems (Schimel and Bennett, 2004). Chapin et al., (1993) showed that organic N also can be important N source in particular terrestrial biomes.

After uptake by plants, N is extensively recycled through the ecosystem by litter production – decomposition – mineralization cycles. The main process that returns N from the biologically available pool back to the atmosphere is denitrification, and at the global scale the N cycle is in equilibrium (Rosswall, 1981). One of the important processes of N redistribution between different ecosystems is N deposition. Deposition originates mainly from dust transport (dry deposition) and wash out of inorganic N from the atmosphere (wet deposition).

In summary, the atmosphere, water bodies and soils contain large amounts of N, but the turnover rates of these reservoirs are extremely slow and most of the biologically available N originates from biological N fixation and recycling. This is

reflected in the N limitation for biological processes that is often found in natural ecosystems (Table 1.1; Vitousek and Howarth, 1991).

Table 1.1 Main reservoirs and turnover times for global N cycle. Values are in Tg N (10^{12} g). (Adapted from http://www.ess.uci.edu/~reeburgh/)

	Reservoir (Tg N)	Turnover (y^{-1})
Atmosphere (N_2)	4×10^9	10^7
Atmosphere (N_2O)	1.4×10^4	100
Sediments	5×10^6	10^7
Ocean (dissolved N_2)	2.2×10^7	1000
Ocean (inorganic)	6×10^5	
Soil	9.5×10^4	2000
Terrestrial biomass	3.5×10^4	50
Marine biomass	4.7×10^2	0.1-1

1.2 Anthropogenic impact on global N cycle

Humans have a continuous impact on the environment. Nowadays it is commonly accepted by the scientific community that human-induced changes, such as changes in the atmospheric composition and land-use, affect global as well local climatic conditions (IPCC, 2007). The N cycle has also been significantly influenced by humans since the early 20th century, with the development of the chemical production of N fertilizers (Haber-Bosch process, 1910). Widespread application of fertilizers continues to alter the natural N cycle. In addition to the huge increase in N fertilizer use, humans have significantly increased the burning of fossil fuels which releases gaseous N to the atmosphere and therefore affects the N cycle. Consequently, since 1950s, human activity has led to natural N fixation rates being doubled and this trend is predicted to increase due to increasing demand for energy and food in the future (Galloway, 1998). The above mentioned processes have led to a sharp increase in the concentration of ammonium and N_2O/NO_X gases in the atmosphere and, subsequently, N deposition across the globe. Nowadays scientists have more and more evidence of N saturation in terrestrial ecosystems, especially in industrialized areas

(e.g. Europe and North America; Vitousek et al., 1997; Galloway and Cowling, 2002). The influence of the altered N cycle on the carbon and water cycles is still not completely clear, partly due to dual feedbacks between the cycles and partly due to the complexity of the processes.

1.3 Dry land perspective

Dry land ecosystems cover a significant area of the Earth's land surface (~30%) and are expected to increase in area due to desertification and climate change (Schlesinger et al., 1990; Reynolds et al., 2007). Recently, desertification of dry lands drew the attention of the United Nations and 2006 was designated as "International Year of the Desert and Desertification." With regards to the Mediterranean basin, the last IPCC report predicted an increase in warming and drought probability for the entire region (IPCC, 2007), which may exacerbate the degradation of dryland areas in the region.

While in dryland ecosystems human impact is expressed in accelerating land degradation (http://lada.virtualcentre.org/), on the global scale, the increase of the carbon dioxide concentration in the atmosphere, since the end of 19th century has the potential to have a pronounced effect on the global climate (IPCC, 2001). Forest planting or afforestation of dry land ecosystems can be used as both a carbon sequestration and land restoration/desertification combating tool (Pacala and Sokolow, 2004).

Important consequences of afforestation, the shift to tree-dominated ecosystems and an increase in aboveground biomass, include an increase in ecosystem carbon storage and a change in biomass distribution and ecosystem activity patterns (Grünzweig et al., 2003; Thuille and Schulze, 2006; Grünzweig et al., 2007). Planting forests on "native" shrubland in semi-arid climate regions possibly has affects on N cycle and turnover. The N in turn largely controls the ecosystem productivity and carbon sequestration by plants. These mutual feedbacks, however, have been insufficiently investigated to date. The expansion of the area covered by vegetation or the plant area index (the ratio of plant to land surface area) may change the overall nutrient distribution, for example Gallardo, (2003) showed increasing concentration of soil organic matter and mineral N in soil nearby an isolated tree. This increase

indicates the development of tree mediated fertility islands similarly to shrub originated ones in the deserts (Schlesinger et al., 1996). However increase in trees density possibly will have an opposite effect, and may reduce the N availability by inhibition of the soil microbial processes and increase of the N uptake from the soil pools (Hart et al., 1993).

An increase in N demand of the ecosystem following the increase in carbon pools may enhance rates of N cycling in order to meet the trees' requirements. The soil N cycle is driven by microbial activities, which is controlled partly by carbon and water availability (e.g., Zhang and Zak, 1998; Kelliher et al., 2004; Ford et al., 2007), and partly by abiotic factors (e.g., Hoyle et al., 2006). Understanding the interplay between biotic and abiotic controls of N cycling in semi-arid ecosystems is crucial for an understanding of the N flow through the system and by this the ecosystem functioning.

In Israel, since the beginning of the 20th century, an ongoing process of afforestation has resulted in more than 90000 ha of planted forest, being nearly 70% of the total (132000 ha) forested area in Israel (FAO, 2001). The common tree species used for planting in Israel is Aleppo pine (*Pinus halepensis* Mill.), which was chosen because of its drought tolerance and Mediterranean origin (Bonneh, 2000).

1.4 Nitrogen cycle in semi–arid ecosystems and afforestation

Interplay between N and water limitation commonly occurs in semi-arid ecosystems (Hooper and Johnson, 1999). This is reflected in low productivity and ecosystem plant community structure with a predominance of dwarf shrubs and annuals (Krueger-Mangold et al., 2004; Borgogno et al., 2007). The afforestation of dry lands by pine species can significantly increase the water use efficiency and carbon sequestration potential of those ecosystems (Grünzweig et al., 2003; Maseyk, 2006). The change in land cover following afforestation, with the increase in biomass and consequently N demand, may also influence the N cycle in the new ecosystem.

Firstly, ecosystem N input and output may change. An increase in N inputs can come from additional N deposition on the leaves due to the increase in leaf surface area, which is then washed out to the soil by rain or taken up directly by the foliage (Lovett et al., 2000). The N inputs through biological N fixation, in contrast to the rates

of deposition, may decrease as result of biological soil crust destruction during afforestation process and slow rates of its recovery (Evans and Belnap, 1999). Afforestation may also change the amount and patterns of gaseous N emissions from the soil for example woody vegetation encroachment has resulted in an increase of nitric oxide emissions in Texas (Martin et al., 2003), while afforestation caused a reduction of the soil emissions in Costa Rica (Reiners et al., 2002).

Secondly, the internal cycle of N in the ecosystem and soil-plant interactions can be influenced. A change in the amount and quality of the litterfall with a change of dominant plant type was shown to have influenced litter decomposition rates and mechanisms of the N return to the soil pool both in temperate forests (Aber et al., 1991) and across the globe (Knops et al., 2002). Pine leaf litter usually has higher a C/N ratio and slower decomposition rate compared to the shrub and annuals litter (Arianoutsou and Radea, 2000; Garcia-Pausas et al., 2004).

The soil N processes are affected as well, Chen et al., (2006) showed that net N mineralization increased in fenced pine plantation relative to the unfenced (grazed) one. Oppositely, Goberna et al., (2007) showed that *P. halepensis* planted on former maquis land reduced soil basal respiration and enzymatic activity in afforested soils. Therefore it is more likely that effects on the specific N-cycle processes depend on the plant type and time scale of the land-use change. For example, soils from a young pine plantation in central Oregon exhibited similar N mineralization rates in mineral soil and higher in the litter layer than those of an old plantation (Kelliher et al., 2004).

Finally, the main mechanism of the inorganic N removal from the soil, microbial and plant uptake depends on plant type, and therefore the effect of afforestation on soil inorganic N dynamics will depend on the makeup of the pre- and post-planting plant communities (Moro et al., 1996; Parfitt et al., 2003). Overall, the land-use change induces changes in N cycle rates, for example increasing nitric oxides emissions in successional forest compared to older one, leads to an increase in ecosystem productivity and N use efficiency in conifer dominated forests and results in an increase of N retention in soil (Fassnacht and Gower, 1999; Erickson et al., 2002; Templer et al., 2005).

1.5 Litter decomposition

The soil inorganic N pool is the only pool available for plant requirements, although organic N was proposed to be an important part of the N supply for plants in arctic regions (see Chapman et al., 2006 and references therein). The rates of addition of new N into the soil inorganic pool from atmospheric deposition are much lower (5-32%) than N intra–system recycling by the coupled decomposition-mineralization processes (Waring and Schlesinger, 1985). The controls on N recycling in the soil and on N availability for plants are solely microbial driven.

The first stage of the recycling process is decomposition of the litter and the subsequent mineralization and release of inorganic N back into the soil pool, where it becomes available to plants (Berg and Laskowski, 2005a). Litter decomposition is a slow process and during the initial stages litter usually immobilizes N from the soil pool, and only thereafter slowly releases N to the soil pool (Berg and Laskowski, 2005b). In the case of the biological decomposition of litter, litter quality, or the C to N ratio plays an important role in the control on decomposition rates. Semi–arid shrubland litter usually have relatively low C/N ratios and decomposition of such litter can be fast compared to pine litter (Arianoutsou and Radea, 2000; Regina, 2001; Bernal et al., 2003; Prescott, 2005; Quideau et al., 2005; Schimel and Hattenschwiler, 2007). However, in arid and semi–arid systems litter decomposition can result from abiotic photochemical processes and in this case the litter quality will have no effect on decomposition rates (Austin and Vivanco, 2006).

1.6 Mineralization and nitrogen availability

N mineralization in semi–arid ecosystems usually follows patterns of water availability. During the summer drought, mineralization rates are extremely low and are possibly induced by dewfall and water absorption by the soil (Agam and Berlinder, 2004). After rewetting, however, mineralization rates can be high and are controlled mainly by temperature (Dalias et al., 2002). During mineralization, large organic molecules are broken down to smaller parts by microbes and these then enter the soil labile pool (Schimel and Bennett, 2004; Schimel and Hattenschwiler, 2007). Mineralization therefore is the main regulatory process on plant N availability (Binkley and Hart, 1989). An additional regulatory process on N availability for plants is plant -

microbial competition for inorganic N. In the past, one of the main assumptions in ecological research was that plants is poor competitors for N, but there is now increasing evidence that plants can out-compete microbes for N (Johnson 1992; Hodge et al., 2000; Korsaeth et al., 2001; Cheng and Bledsoe, 2004). Finally, the oxidation of reduced forms of N (e.g. ammonium) to more labile nitrate and the subsequent leaching of nitrate out from the root zone is an important process that both regulates N availability and contributes to the contamination of groundwater, mainly in mesic ecosystems (Matson et al., 1999; Zechmeister-Boltenstern et al., 2002; Walvoord et al., 2003; Austin et al., 2004).

1.7 Nitrification

Nitrification is the only process that converts reduced forms of N (e.g. ammonium) into more oxidized, mobile nitrate, and therefore plays an important role in controls on both ecosystem productivity and the global terrestrial N cycle. N additional process that accounts for ammonium oxidation, anoxic ammonia oxidation (ANAMMOX), although important in the aquatic environments was not shown to take place in soils (Jetten, 2001). Shown below are the main steps of nitrification, ammonia oxidation, nitrite oxidation, and the groups of microorganisms that involved in these reactions:

$$NH_4^+ + O_2 + H^+ \rightarrow \{NH_2OH\} + H_2O \rightarrow NO_2^- + 5H^+ \text{ (AOB)}$$

$$NO_2^- + H_2O \rightarrow NO_3^- + 2H^+ \text{ (NOB)}$$

Two different groups of microorganisms are involved in the nitrification process, Ammonia Oxidizing Bacteria (AOB) and Nitrite Oxidizing Bacteria (NOB). These two groups of microorganisms have different tolerance for environmental conditions such as temperature, pH and soil water content. Overall, nitrite oxidizers are more sensitive to temperature and drought stress, while the ammonia oxidizers more sensitive to the low pH (Stark and Firestone, 1995; Avrahami et al., 2003; Avrahami and Conrad, 2005).

The first step of nitrification, ammonia oxidation, is carried out by three genera within the β-proteobacteria: *Nitrosomonas, Nitrosospira and Nitrosococcus* (Purkhold

et al., 2000) and is considered as a rate-limiting step of the nitrification process (Kowalchuk and Stephen, 2001). The second step of nitrification, the oxidation of nitrite into nitrate, carried out by four different genera: *Nitrobacter*, *Nitrococcus*, *Nitrospira,* and *Nitrospina* (Hagopian and Riley, 1998) and is widely accepted to be a fast process. Indeed, an intermediate of the nitrification process, nitrite (NO_2^-), rarely accumulates in terrestrial ecosystems (Paul and Clarke, 1989; De Boer and Kester, 1996).

In terrestrial ecosystems nitrite accumulation was measured only following human interference, for example nitrites accumulated in forest and pasture soils when a nitrite oxidation inhibitor (nitrapyrin) was added (Shen et al., 2003). Additionally, when environmental factors such as N fertilizer load, organic matter addition and water limitation in soils were manipulated, substantial quantities of nitrite accumulated (Chapman and Liebig, 1952; Pagel, 1979; Hamilton and Lowe, 1981; Burns et al., 1995; Stevens et al., 1998; Shen et al., 2003). Larsen, (1971) showed nitrite accumulation in laboratory experiments due to manipulation of soil pH, and related this accumulation to different pH optima of the two nitrifying bacteria (AOB and NOB). The main reason for accumulation of nitrite in well-aerated environments was proposed to be a partial nitrification process where ammonia is oxidized into nitrite but NOB is either absent or inhibited (Smith et al., 1997).

1.8 Nitrogen Oxides emissions

Associated with changes in land cover, or land management practices, are changes in trace-gas emissions, which should be clarified. One of the possible effects of these changes, the environmental benefits from an increase in carbon sequestration, may be negated by an associated increase in emissions of other greenhouse gases, such as nitric oxide (NO) or nitrous oxide (N_2O) (Smith and Conen, 2004). In Texas, woody vegetation encroachment has resulted in an increase in NO emissions from 0.9 ng m^{-2} s^{-1} to 18 ng m^{-2} s^{-1} (all emission values are in terms of mass of N) (Martin et al., 2003). However, afforested sites in Costa Rica have been shown to have lower NO emission rates compared to pastures and banana plantations (0.9, 8.9, 15.6 kg ha^{-1} y^{-1} respectively; Reiners et al., 2002).

Emissions of NO and it's conversion to NO_2^- are important in regulating chemical processes in the atmosphere through ozone destruction and formation sequences (Crutzen, 1979; Chameides et al., 1992; Crutzen, 1995; Levine et al., 1997; Eq. 1.1-1.3). The cycle of reactions (Eq. 1.1-1.3) is driven by sunlight, with the initial step being the photolysis of NO_2 to produce NO and a triplet state of oxygen atom O (^{3}P). The O (^{3}P) reacts rapidly with oxygen to produce ozone and ozone reacts with NO to produce NO_2 (Cape, 2002).

NO is a highly reactive gas and there is a rapid chemical inter-conversion with NO_2, therefore both species are key catalysts in the reactions that generate and destroy ozone and are commonly referred to as NO_X ($NO + NO_2 = NO_X$) (Ludwig et al., 2001). Thus, the ambient mixing ratio of NO_X creates the threshold which determines whether ozone is created or destroyed (Levine et al., 1996; Lindesay, 1997; Meixner and Yang, 2006). Most tropospheric ozone is produced in the reactions between non-methane hydrocarbons and oxides of N and by the oxidation of methane by the OH radical in the presence of NO_X. In low NO_X environments the oxidation process leads to ozone destruction. The removal of NO_X is through a series of photochemical reactions that produce nitric acid; this is an important component of acid rain and acts as a source of N deposited from the atmosphere (Logan, 1983; Eq. 1.4-1.6).

$$NO_2 + h\nu\ (8 < 400\,nm) \rightarrow NO + O(^3P) \quad (1.1)$$

$$O(^3P) + O_2 \rightarrow O_3 \quad (1.2)$$

$$O_3 + NO \rightarrow NO_2 + O_2 \quad (1.3)$$

$$O_3 + h\nu\,(\lambda < 310nm) \rightarrow O_2 + O(^1D) \quad (1.4)$$

$$O(^1D) + H_2O \rightarrow 2\,OH \quad (1.5)$$

$$NO_2 + OH \rightarrow HNO_3 \quad (1.6)$$

According to current knowledge, NO (as well as N_2O) is produced in soils nearly ubiquitously and therefore soil emissions constitute a continuous background flux of NO to the atmosphere (Davidson, 1991). Global inputs of NO_X to the atmosphere are estimated to be between 33 and 50 Tg y^{-1}, with soil contributing between 18% and

40% of the total emission and anthropogenic sources accounting for approximately 50% (~21 Tg), with a relatively large uncertainty of 4 to 10 Tg N y^{-1} (Davidson and Kingerlee, 1997; IPCC, 2001). As can be seen from these numbers, there is still large uncertainty in estimations of NO_X fluxes from the terrestrial biomes. However, despite considerable uncertainties, there is substantial evidence that soil emissions make a significant contribution to the tropospheric NO_X budget even in industrialized regions of the globe (Williams, 1992; Davidson and Kingerlee, 1997). Emissions from semi-arid and arid lands, in particular, contribute to uncertainties because of a very small number of NO_X flux measurements. A recent review by Meixner and Yang, (2006) identified only 13 studies on natural semi–arid and arid ecosystems (annual precipitation below 400 mm), including a single study from the Mediterranean region. If current and future efforts to reduce NO_X emissions from vehicles and fossil fuel burning are successful the relative importance of biogenic emissions will grow considerably in the future. While biogenic emissions from natural systems are part of the natural background levels, the increase in the use of fertilizers and in land use changes may significantly influence the global NO_X budget.

The net flux of NO from the soil is influenced by soil N content, temperature, moisture and texture (Martin et al., 1998; Ludwig et al., 2001; Skiba et al., 2006). Soil cover has a regulatory role in determining the flux of NO into the atmosphere, since NO emitted from vegetated soils is converted into NO_2 by O_3 within most plant canopies and can then be re-deposited on the vegetation surface (in gaseous form) (Ludwig et al., 2001). In addition, direct (stomatal) uptake of NO_2 by plants is well known, whereas for NO this uptake pathway is generally negligible (Hereid and Monson, 2001; Sparks et al., 2001). Finally, recent studies have shown that NO plays an important role as a signalling molecule in plants and affects a broad range of plant physiological activities, from stomatal closure to regulating mitochondrial and chloroplast functions (Stöhr and Ullrich, 2002; Neill et al., 2003; Shapiro, 2005).

1.9 Aims of the study

This research addresses primarily the question of what changes in the overall ecosystem N budget and internal N cycling are associated with the observed large increase in ecosystem carbon stocks in pine afforestation of a semi–arid shrubland. This is based on testing several hypotheses and the specific objectives were:

1. To test the hypothesis that conversion of shrubland to forest in the semi-arid regions enhances N deposition and fixation by soil, and accelerates N mineralization and turnover.
2. To test the hypothesis that temporal synchronization in recovery from seasonal drought stress can improve N availability and demand in the semi-arid forest ecosystem.
3. To constructing a detailed ecosystem-scale N budget for the shrubland and afforestation system used in this study.
4. To identify inter-annual variations in N cycling in the shrubland and forest ecosystem, including: atmospheric N deposition, N fixation by soil, N requirements for growth and litter decomposition processes.
5. To identify controls on nitrification during rewetting of soils at the start of the wet season.
6. To valuate changes in N losses via NO emission from soils in going from shrubland to forest ecosystems.

Chapter 2

2 Materials and Methods

2.1 Site description

This study was conducted in the Yatir Forest (~2800 ha). A pine plantation established at the edge of Northern Negev desert, and surrounding shrubland, (31º20'49.2''N, 35º03'07.2''E) at a mean altitude of about 650 m a. s. l. Mean annual precipitation at the Yatir forest is 280 mm (last 30 years). Trees are mainly *Pinus halepensis* Mill. (Ne'eman and Trabaud, 2000) planted during 1965 - 69, by Jewish National Fund. The surrounding native vegetation is dominated by the dwarf shrub *Sarcopoterium spinosum* (L.) (Litav and Orshan, 1971), and patches of herbaceous annuals and perennials (total vegetation height of 30 - 50 cm). The Mediterranean climate of the Northern Negev is characterized by an extended dry period between May and October or November. Precipitation occurs only between October or November and May, with high inter- and intra-annual variations. Mean annual temperature is ~18°C, with average maximum and minimum of 32.3°C and 6.9°C, respectively (over the study period). The soil is a Lithosol (FAO) with pH 7.8±0.2 (water), above chalk and limestone with a depth of 0.2-1 m. Soil bulk density is 1.65×10^{3} kg m^{-3}. The particle size distribution of the soils is silty - clay (USDA). The site includes a 19 m flux tower for ecosystem-level measurements of net ecosystem exchange (NEE) of carbon dioxide, energy, and water vapor, as well as meteorological measurements.

2.2 Dry and bulk deposition, collection and measurements

The determination of the rate of N input to the systems by dry deposition, was assessed by using boxes (40×32×10 cm), filled with glass beads (16 mm diameter) in one layer, according to Offer et al., (1998). Dry deposition was collected bi-monthly by manually brushing the glass beads into paper bags. Inside the afforestation, boxes were placed at the flux tower site in groups of five, each with the distance between boxes of around 1 - 2 m and >200 m between different groups. The boxes were placed below the tree canopy. In the shrubland, deposition was collected at three sites by

groups of five boxes on the roofs of structures bordering the afforestation in three different directions, about 2 - 3 m above the canopy of the shrubs at each site. Total-N content of the samples was determined with a CHN-O elemental analyzer (EA1108 Carlo-Erba, Milan, Italy).

Bulk deposition (wet and dry deposition) was collected in the wet season over two years, with funneled bottles (funnel diameter 11 cm) at the same locations as dry deposition collectors. Five bottles at each site were installed, total 15 bottles for the afforested sites and 15 bottles for shrubland sites. Funnels on the bottles were covered with a plastic mesh (5 mm) to prevent the entrance of insects. A small styrofoam ball was introduced to each funnel to prevent evaporation. After each rain event, samples were collected to sterile plastic bottles, transported to the lab, centrifuged to remove solids, and stored at -20°C until analysis for inorganic N content.

Two different parameters were calculated from the deposition measurements. Firstly we calculated the N deposition to the different sites. Secondly we calculated how much inorganic N was added to the soil by direct bulk deposition to the afforested plots. By the second calculation it was desired to check whether N deposited could explain the changes in the concentration of different N forms in the soil. The inorganic N addition (mg kg^{-1}) to the soil by bulk deposition was estimated according to:

$$Nadd = \frac{Nbulk \times Rain}{Soil} \tag{2.1}$$

where: *Nadd* - inorganic N addition (mg kg^{-1}), *Nbulk* - concentration of N in the bulk deposition (mg N l^{-1}), *Rain* - bulk deposition (l m^{-2}), *Soil* - kg soil in 0 - 10 cm layer (kg m^{-3}).

Secondly, the dry deposition to the ecosystems was calculated by extrapolation from the results obtained by the collection boxes on an area basis. Total bulk deposition was calculated accordingly to:

$$Nbulk = [N]rain \times Rain \tag{2.2}$$

where: *Nbulk* - Bulk N deposition (g m^{-2}), *[N]rain* - N concentration (mg l^{-1}) in rain and *Rain* is amount of rain (l m^{-2}).

2.3 Soil sampling

Three representative plots of approximately 1 ha in the central section of the afforestation and shrubland were chosen. At each plot, five random replications of mineral soil samples were taken from a depth of 0 - 10 cm using a 50 mm diameter corer (BenMeadows Company, Canada). Soil samples were collected inside the Yatir forest (*forest*) and in the surrounding shrubland. The shrubland sites were divided into two microsites: (1) under shrub canopy (*shrub*) and (2) at inter-shrub interval soils (*bare*).

The samples were brought to the laboratory on the same day of sampling, and were stored at 4°C. We are aware of the problems associated with soil conservation, to avoid possible changes in inorganic N composition and concentrations, the samples for inorganic N extraction were kept in the refrigerator for as little time as possible, not longer than 7 - 10 days. The samples for further experiments were kept for a longer time (up to 18 - 20 days). Depending on the conditions of the soils in the field, the samples with gravimetric water content (GWC) less than 8% were in the refrigeration longer and the samples from the wet period, where the water concentration was high (up to 25%) were kept in the refrigeration no longer than one week.

Samples for measurement of biogenic NO_X emissions (Chapter 5) were collected during October 2005, February 2006, and May 2006. Three sampling periods represented the main seasons; they differ in both water content and temperature (see section 2.8 for details). The samples were then brought to the laboratory on the day of sampling, sieved (2 mm), bulked, homogenized, and stored at 4°C. The soil samples from October 2005 and May 2006 were practically air-dried. The October 2005 soils had GWC of 3.8, 4.8, and 4.8% for *bare* and vegetation-covered soils (*forest* and *shrub*) respectively. The soil samples from May 2006 had a GWC of 5.4, 7.2, and 7.6% for *bare*, *shrub,* and *forest* soils, respectively. Soils from February 2006 had a GWC of 16.5, 20.8, and 22.2% for *bare*, *shrub,* and *forest* soils, respectively. The February 2006 samples were slightly dried prior to storage. Samples used for incubations were delivered under cool conditions (approx. 5°C) to Mainz, Germany. The *in situ* soil water content and temperature were measured at the Yatir flux tower site (Grünzweig et al., 2003). Temperatures were measured with T-type thermocouple

sensors placed at a 6 cm depth. Sensors were placed under the tree canopy and in a clearing to represent *bare*- and canopy-covered soils (i.e., *forest* and *shrub*). The volumetric water content (VWC) of the soil was measured with a Time-Domain Reflectrometry (TDR) sensor (IMKO, Germany). Sensors were placed at the same plot as the temperature sensors, at 5 cm depth. The average water content measured by TDR sensors was in good agreement with destructive measurements of the soil water content at the forested and shrubland sites (0 - 10cm soil layer, core sampling; Raz-Yaseef, N., unpublished data). Both water and temperature were measured with half-hour resolution and have been averaged to yield daily mean values.

2.4 Net N-mineralization *in situ*

In situ net N-mineralization was measured by the undisturbed buried core method (Hart et al., 1994), assuming that nitrogen mineralization rates inside and outside of cores was similar. Plastic caps covering the cores had holes in order to reduce disturbance and the entrance of direct rainfall while allowing for gas exchange. Five paired samples (within 20 cm of each other) at each plot (*forest, shrub* and *bare*) were taken, one sample for the determination of initial conditions and the other for *in situ* incubation. The second sample was taken into a 15 cm long butyrate plastic liner, covered by a cap, and returned to the soil. At the end of the incubation period, cores were recovered and taken to the laboratory and stored at 4°C prior to processing. A new set of cores was installed immediately after sampling, at new randomly selected locations within the plot, for the next incubation period. At the shrubland plots, mineralization was estimated both in *shrub* and *bare* soils, five random samples were taken at each microsite. The incubation periods varied depending on the season: two to three months during the wet (December - May) season, and up to five months during the dry season (June - November). The differences in incubation time between the two seasons were due to very different conditions of the soil at dry and wet seasons. The dry season was characterized by a very low average GWC (5 - 8% from the middle of June to the first rain events at end of October - November). Due to these conditions we assumed low net N-mineralization rates. The wet season, characterized by a GWC of 10 - 25%, we therefore assumed that changes in the net N-mineralization rates could be

rapid. Net N-mineralization rates were estimated using changes of inorganic nitrogen concentrations in the soil core, after incubation period according to:

$$Nm = \sum Nfinal - \sum Nin \quad (2.3)$$

where: ***Nm*** - Net N mineralization rates *in situ* in μg N g^{-1} sdw week $^{-1}$, ***ΣN*** - Total concentration of all inorganic nitrogen forms at the end (***N final***) and at the beginning (***N in***) of incubation in μg N g^{-1} sdw, and "sdw" is the soil dry weight.

2.5 Soil extraction and mineral nitrogen analysis

On the day of processing soil samples (including cores), samples were sieved to <2 mm aggregate size or manually homogenized (when water content disallowed the sieving). Large roots and stones were removed manually. Four replicates of 5 gr sub-samples from each sample were weighed into 25 ml of 2N KCl solution (soil to solution ratio 1:5) and extracted for one hour with continuous shaking at 180 rev min^{-1}. Extracts were filtered on pre-washed filters (Whatman no. 42 paper, Whatman, UK) and stored at -20°C prior to inorganic N analyses. Inorganic N concentration was determined by colorimetric methods: ammonium-N ($N\text{-}NH_4^+$) by silicate-hypochlorite method (Bower and Holm-Hansen, 1980); nitrite-N ($N\text{-}NO_2^-$) according to (Strickland and Parsons, 1968); nitrate-N ($N\text{-}NO_3^-$) by the dual-wavelength ultraviolet spectrophotometric method after Norman et al., (1985). The detection limits for the inorganic N concentration determination were: $N\text{-}NO_2^-$: 0.14 μmol l^{-1}, $N\text{-}NH_4^+$: 0.7 μmol l^{-1} and $N\text{-}NO_3^-$: 3.6 μmol l^{-1}. All colorimetric measurements were conducted on a double beam UV/VIS Cary spectrophotometer (Carry100, Varian, Palo Alto, CA, USA). Soil GWC was determined by drying samples in an oven at 105°C for 48 h, and measuring change in soil weight.

2.6 Total Carbon and Nitrogen content of litter and soils

Total carbon, total N concentrations and isotopic analysis of soil and litter were determined on oven dried (50 - 60°C for 48 h) and mechanically ground <250 μm samples. Two replicates from each sample of ~0.3 - 4 mg were weighed into 3×5 mm

tin foil capsules (Elemental Microanalysis Ltd., UK) and combusted in an elemental analyzer (Carbo Erba 1108; Carlo Erba, Italy, precision ±0.5%).

2.7 Laboratory incubations

2.7.1 Incubations for estimation of nitrite accumulation rates

Soil for laboratory incubations, were collected from the same field plots mentioned above (section 2.3), at each plot five random replicates of mineral soil (0 - 10 cm) were collected. The laboratory incubations for *forest* plots proceeded with soils from: the end of the dry season (October 2005), after the first rain events (December 2005), and the middle of the wet (period of ecosystem activity) season (February 2006). The laboratory incubations for shrubland plots (*shrub* and *bare* microsites) were from soils of October 2005 and February 2006 only. The soil samples from each of the three field plots were bulked, homogenized, and mixed well. Slurries with 1:10 soil to mineral media were set up in Erlenmeyer flasks. Slightly modified Potential Nitrification assay with the same medium, but, without chlorate addition were used. The principles of such laboratory incubations have been discussed in more detail elsewhere (Belser, 1979; Belser and Mays, 1980; Hart et al., 1994). We choose to use slurry and not jar incubations in order to check the potential activity of the nitrifiers in the soils without possible adaptation (due to long incubation time) of the soils to the conditions of the laboratory which is particularly difficult when using the jar incubation method. The medium contained 0.25 mM $(NH_4)_2SO_4$ and 1 mM K_2HPO_4 at pH of 7.8. The incubations were performed on rotation shaker with temperature of 28±1°C, and rotation speed of 150 rev min^{-1}, to achieve fully aerobic conditions. The slurries were incubated in triplicate for ~90 h with periodical sampling. Samples were centrifuged at 14000 rev min^{-1} to remove soil particles then immediately stored at -20°C until analysis for nitrite, nitrate, and ammonium (see above, section 2.5).

2.7.2 Incubations for potential NO emission measurements

The procedure for soil incubation and laboratory NO flux measurements has been described in detail elsewhere (Otter et al., 1999; van Dijk et al., 2002 and van Dijk and Meixner, 2001). In short, the system for laboratory incubations contains five

parallel flow-through chambers (0.9 l volume), four for soil samples, and one (without soil) for reference. Micro-fans in the head-space of the chambers ensured well-mixed conditions within the incubation chambers. NO was measured by a chemoluminescence trace gas analyzer CLD780TR (Ecophysics AG, Switzerland). In addition, the evaporation rate was measured by using an infrared H_2O/CO_2 gas analyzer (BINOS, Rosemont). Laboratory incubations were made using ~100 g sub-samples of the bulked soil samples. Before incubation, the sub-samples were wet with deionized water to the individual soil's water-holding capacity, and pre-incubated at room temperature (~24°C) for ~72 h. The pre-incubation was performed to standardize conditions within the soil sub-samples, since the soils had differing water contents and had experienced different refrigerating periods during storage. For estimating zero-activity, we performed incubations with autoclaved soils.

The net NO release from soil samples was calculated from the difference between the NO mixing ratio at the outlet of the reference chamber and the outlet of each incubation chamber. The net NO release rate (J_{NO}, in ng kg^{-1} s^{-1}) is calculated according to:

$$J_{NO} = \frac{Q}{M_{soil}} \left(m_{NO,out} - m_{NO,ref} \right) \frac{M_N}{V_m} \times 10^{-3} \qquad (2.4)$$

where: Q is the flow rate through cuvette (4.17×10^{-5} m^3 s^{-1} or 2.5 l min^{-1}), M_{soil} is the soil dry mass (kg), and $m_{NO,ref}$ and $m_{NO,out}$ are the NO mixing ratios (in ppb or 10^{-9}) at the outlets of the reference and the incubation chambers, respectively. The conversion factor (ppb to ng m^{-3}) is defined by $M_N/V_m\times10^{-3}$, where M_N is the molecular mass of N (kg $kmole^{-1}$), and V_m is the molar volume (m^3 $kmole^{-1}$).

The release of NO from the soil results from microbial NO production and NO consumption, which operate simultaneously (Conrad, 1994; 1996). Consequently, the NO release rate (J_{NO}) observed during incubation (eq. 2.4) is always a net release rate. If NO consumption overrides NO production in the soil sample, then J_{NO} becomes negative. According to eq. (2.4), this only occurs if the incoming NO mixing ratio (which is equal to $m_{NO,,ref}$) exceeds the headspace NO mixing ratio (which is equal to $m_{NO,,out}$ due to well-mixed conditions within the incubation chamber). Remde et al.,

(1989), Ludwig et al., (2001), and van Dijk and Meixner, (2001) have already shown that there is an experimentally proven linear relationship between the net NO release rate J_{NO}, and the rates of NO production, *P,* and consumption, *k*, so that the measured release rates, J_{NO}, can be described as:

$$J_{NO} = P - k \times m_{NO,out} \times \frac{M_N}{V_m} \times 10^{-3} \quad (2.5)$$

Equation 2.5 implies that the NO production rate *P* is independent of the headspace NO mixing ratio ($m_{NO,,out}$), whereas the first-order NO consumption rate, *k,* is dependent upon it. In order to determine *P* and *k*, we used eq. (2.5) with measured fluxes (J_{NO}, eq. 2.4) from two sets of incubation measurements: $m_{NO,ref}$ = 0 ppb and $m_{NO,ref}$ = 56 ppb, on sub-samples of each soil sample. This allowed us to calculate *P* (in ng kg^{-1} s^{-1}) and *k* (in m^3 kg^{-1} s^{-1}).

We present the results of NO release rates as a function of the Water Filled Pore Space (WFPS). WFPS was determined from (a) the amount of water lost by evaporation from the enclosed soil sample during incubation, and (b) the GWC of the sample (by drying a subsample at 105°C for 48 h):

$$WFPS = GWC \times BD / (1 - BD \times 2.65 \times 10^{-3}) \quad (2.6)$$

where: GWC is the gravimetric water content in %; BD is the bulk soil density (kg m^{-3}), and the particle density of the average mineral (quartz) soil is 2.65×10^3 kg m^{-3} according to Parton et al., (2001).

The temperature response of soil NO release were derived from two sets of NO release rate (each on another set of sub-samples of the same soil) measurements, where the sub-samples were always identically treated except for incubation at 18°C and 28°C.

Finally, eq. (2.5) is extended to describe the net NO release rate and its partitioning (P and k), for each soil sample, as a function of the relevant variables, headspace NO mixing ratio (= $m_{NO,,out}$), $WFPS$, and soil temperature (T_{soil}):

$$J_{NO}(m_{NO,out}, WFPS, T_{soil}) = P(WFPS, T_{soil}) - k(WFPS, T_{soil})\, m_{NO,out}\, \frac{M_N}{V_m} \times 10^{-3} \qquad (2.7)$$

2.8 Upscaling of NO_X emission measurements to the ecosystem scale

NO release rates (J_{NO}), derived from laboratory incubations and parameterized for measured T_{soil}, WFPS, and m_{NO}, were up-scaled to estimate field net NO fluxes (F_{NO}) by applying the field measurements of these parameters. Similar up-scaling has already been reported by Kirkman et al. (2001), and verification of the up-scaling procedure has been repeatedly performed by demonstrating that NO fluxes, measured in the field by the dynamic chamber technique, were in good agreement with those derived from laboratory incubations on soil samples (taken from the top soil of dynamic chambers' enclosures, e.g., Ludwig et al., (2001), van Dijk et al., (2002)). Up-scaling is achieved by applying the modified (see below) algorithm developed by Galbally and Johansson (1989) and subsequently improved by Meixner et al. (1997), Kirkman et al. (2001), Van Dijk and Meixner (2001), Van Dijk, et al. (2002) and Meixner and Yang (2006):

$$F_{NO}(WFPS) = \sqrt{D(WFPS)\, BD\, k}\left(\frac{P(WFPS)}{k} - m_{NOamb}\frac{M_N}{V_m} \times 10^{-3}\right) \qquad (2.8)$$

where F_{NO} is the estimated net NO flux (ng m^2 s^{-1}), BD is the bulk density of soil (kg m^{-3}), k is the NO consumption rate (m^3 kg^{-1} s^{-1}), D is the effective diffusion coefficient of NO in soil (in m^2 s^{-1}) calculated accordingly to Moldrup et al. (2000), P is the NO production rate (ng kg^{-1} s^{-1}) calculated from measured J_{NO} eq. (2.4) using eq. (2.7), and m_{NOamb} is the ambient NO mixing ratio (ppb). 2005 to 2006 field data of the NO mixing ratio (average of 2.39±0.56 ppb) were provided by the Israeli Ministry of Environmental Protection, from measurements at the Gush Ezion air quality monitoring station (distance of ~35 km from our research site; http://www.sviva.gov.il). Since corresponding soil moisture and soil temperature data were calculated as daily means, F_{NO} was up-scaled on a daily basis for all studied soils,

for each sampling season and location (i.e., October 2005, February 2006, and May 2006, *forest*, *bare,* and *shrub* soils).

In contrast to Meixner and Yang (2006), we used in eq. (2.8) a constant NO consumption rate, *k*, (rather than *k*(WFPS)). We estimated *k* values of studied soils, and found that different soils (i.e. *forest*, *shrub* and *bare*) had different *k* values (data not shown). The *forest* soils have average *k* value of 0.67×10^{-5} m^3 kg^{-1} s^{-1} in the WFPS range between 5% and 18%. While *bare* soils have average *k* value of 3.65×10^{-5} m^3 kg^{-1} s^{-1}. We did not succeed to measure any *k* value for *shrub* soils, however, we assume that *shrub* soil represent intermediate stage between fully plant covered *forest* and annuals covered *bare* soils. Therefore we can assume that *k* values for the *shrub* soils need to be in between the measured *k* (1.58×10^{-5} m^3 kg^{-1} s^{-1}). The above mentioned *k* values are consistent with other, literature published *k* values. We used a constant *k*-value of 1.6×10^{-5} m^3 kg^{-1} s^{-1} for the *forest*, *bare,* and *shrub* soils, which was obtained from the report of Otter et al. (1999) for semi-arid savannas, and which is in the range of *k* values for a wide range of soils (2.6×10^{-5} m^{-3} kg^{-1} s^{-1} to 27.7×10^{-5} m^{-3} kg^{-1} s^{-1}; Rudolph et al., (1996); Bollmann et al., (1999); Godde and Conrad, (2000)). For calculation of "gross" F_{NO} we used uncorrected J_{NO} data for fitting (see below), the conversion from the ng kg^{-1} s^{-1} to ng m^{-2} s^{-1} was done using field BD of 0-10 cm soil layer (Ramde et al., 1993; Conrad, 1994).

To describe the dependence of F_{NO} on WFPS at a constant reference soil temperature (T_{ref} = 28°C), we made use of eq. (2.8) to calculate data pairs (F_{NO}, WFPS) from our observations and fitted them similarly to the Meixner and Yang (2006) procedure by a mathematical algorithm (OriginLab Corp., MA, USA):

$$F_{NO}\left(WFPS, T_{ref}\right) = y_{0T_{ref}} + \frac{A_{T_{ref}}}{w_{T_{ref}}\sqrt{\pi/2}} \times e^{b} \qquad (2.9)$$

where $b = -2 \times \frac{(WFPS - X_{cT_{ref}})^2}{w_{T_{ref}}^{\ 2}}$ and from which y_{oTref}, A_{Tref}, w_{Tref}, X_{cTref}, can be derived.

Similar to Van Dijk and Meixner (2001) and Van Dijk et al. (2002), we assumed the dependence of F_{NO} on soil temperature to be an exponential relationship (the general form of temperature dependence for enzymatic processes). To quantify the

relationships, we utilized our measurements on J_{NO} at two different incubation temperatures (see section 2.7.2). The response of the NO release to soil temperature could be directly determined for any given WFPS in terms of Q_{10}, i.e.:

$$Q_{10}(WFPS) = J_{NO}(WFPS, T_{soil} = 28°C) / J_{NO}(WFPS, T_{soil} = 18°C) \quad (2.10)$$

For the up-scaling procedure, we assumed that Q_{10} for F_{NO} (field) is the same as Q_{10} for J_{NO}, (lab) since both are assumed to be driven by the same microbial processes.

Finally, we combined the functional dependence of F_{NO} on soil moisture and soil temperature (eqs. 2.9 and 2.10) and calculated the field net NO fluxes, F_{NO} (in ng m^{-2} s^{-1}), according to:

$$F_{NO}(WFPS, T_{soil}) = y_{0T_{ref}} + \frac{A_{T_{ref}}}{w_{T_{ref}}\sqrt{\pi/2}} \times e^{b} \times \exp\left[0.1 \times \ln Q_{10} \times \left(T_{soil} - T_{ref}\right)\right] \quad (2.11)$$

where parameters as above and *WFPS* is calculated from the field measurements of VWC and field soil BD (see eq. 2.6), T_{soil} is the soil temperature at 6 cm depth, measured in the field, and T_{ref} is the incubation temperature (28°C; see sections 2.3 and 2.7.2).

In order to up-scale the laboratory measurements, we divided the yearly data set (daily averages) of the field soil water content (5 cm depth) and soil temperature (6 cm depth) measured in three different sections. These correspond to three different periods characterized by different field temperatures and WFPS dynamics, as well as by different NO release patterns during the laboratory incubations.

The three time periods that we defined were *dry-rewetting, wet,* and *drying*. The *dry-rewetting* period had two sub-periods: *dry* and *rewetting* periods, which will be referred to as *dry* and *rewetting*, respectively. The first, *dry*, sub-period (20 July to 10 October), was characterized by a stable WFPS of ~14% and a soil temperature well above 20°C (20 to 35°C). The second sub-period (*rewetting*), is the period from the first rain event with consequent drying of soil to almost *dry* period WFPS values. This period lasted from 11 October, when the first rain event took place, to 25 December, when the second significant rain event occurred (after which soils reached ~66%

WFPS). During *rewetting*, WFPS varied from 14% to a maximum >48% and, following drying of the soil, down to 26% again. The soil temperature during *rewetting* decreased from >20°C to ~15°C. The *wet* season ranged from December 25, 2005 to April 17, 2006, during which the WFPS remained quite high, between 40% and 73%. The mean daily soil temperature was generally 10°C to 15°C, with a minimum of ~7°C. The *drying* season was taken to be from April 18, 2006 to July 19, 2006, accordingly to changes in WFPS and soil temperature. During the *drying* season, the soil temperature increased to >20°C and WFPS decreased back to the *dry* sub-period values of ~14% and remained stable.

We used the seasonally determined J_{NO}-values derived from the laboratory incubations of the soil samples from October 2005 (*dry-rewetting*), February 2006 (*wet*), and May 2006 (*drying*) and linearly interpolated (weighted linear interpolation) between the seasons to estimate the transitions between seasons. The soil temperature measurements from soils at a forest clearing were used for up-scaling the *bare* NO emissions, and soil temperatures measured under the tree canopy were used for up-scaling *forest* and *shrub* NO emission (there were no continuous temperature measurements in the shrubland). The ambient NO mixing ratios we used for upscaling were provided by the Israeli Ministry of Environmental Protection and were 2.66±0.67 ppb for the *dry-rewetting* season, 2.42±0.61 ppb for the *wet* season, and 2.14±0.33 ppb for the *drying* season, respectively. Free surface area, s_i, was corrected for rocks, stamps, and tree stems (the latter two were insignificant) from 10 random transect walks during which a one-meter resolution description of the soil cover (i.e., free soil, rock, stamps and stems) was done. The sampling was performed on four, 1 ha size, representative plots in the forest and four plots in the shrubland.

2.9 Error estimation (potential NO emission measurements)

We found that autoclaved soils release NO at a rate of 0.05 ng kg^{-1} s^{-1} with a random deviation of 0.02 ng kg^{-1} s^{-1} at all WFPS. Therefore, we considered a NO release of 0.10 ng kg^{-1} s^{-1} (release rate of autoclaved soils plus three standard deviations from the mean release rate, with a confidence interval of 99.7%) as the experimentally derived detection limit for J_{NO} of our incubation technique. Furthermore, the error of the NO release rate measurements was determined

experimentally by incubation of soils in four replicates. The mean standard deviation of the NO release rate in four replicates was found to be 0.03 ng kg^{-1} s^{-1} for all WFPS, i.e., lower than the experimentally derived detection limit of J_{NO} as well as the error that would result from the detection limit of the NO mixing ratio by the chemoluminescence analyzer used. Based on these observations, we consider ±0.05 ng kg^{-1} s^{-1} as a conservative estimate of the overall experimental error of J_{NO}.

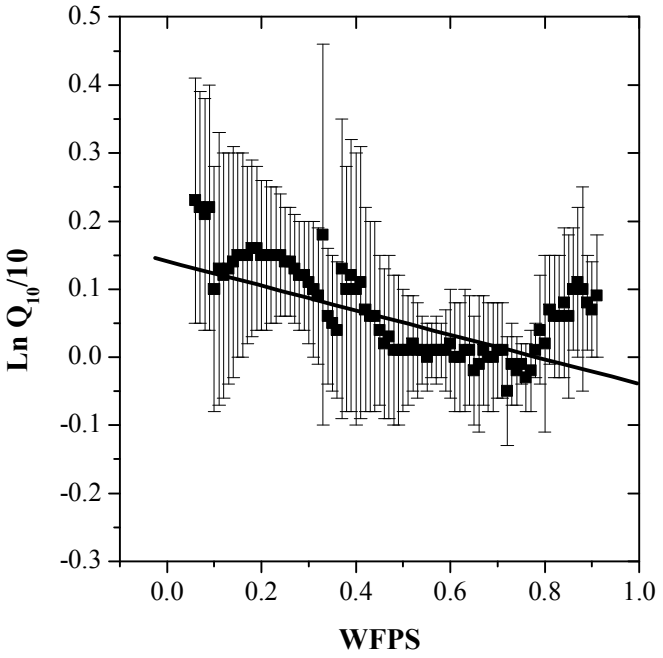


Figure 2.1 Correlation between $LnQ_{10}/10$ and WFPS. The presented results are from laboratory incubations with all types of soils from studied ecosystems (i.e. *forest*, *shrub*, *bare*). $r^2 = 0.42$; $p<0.0001$.

2.10 Litter decomposition assay

Litter decomposition assay are described in detail in Grünzweig et al., (2007). Briefly, local litter was placed in litter bags for decomposition in the forest and shrubland sites. Local litter in the forest consisted of needles (C/N ratio = 146.5) collected in litter traps during the 2004-2005 season and of roots (C/N ratio = 61.1) collected from three plots to a depth of 10-15 cm at the end of the dry season in 2005. Local litter in the shrubland consisted of standing leaf litter (C/N ratio = 37.8) and of roots to a depth of 10 - 15 cm (C/N ratio = 34.5) collected from three plots, both under

shrubs and in the inter-shrub microsites, at the end of the dry season of 2005. Initial litter dry mass (dm) was 3.0±0.2 g in all litter bags. Litter was placed in the field for three different time intervals. Litter placed in the field in January 2006 for decomposition of 4 months during the wet season, and in June 2006 for decomposition of 4 months during the dry season. Additional sets of litterbags were placed in the field in June 2006 for decomposition over a 12 month period. Litterbags that were placed in the field and immediately retrieved served as a control. The net N change in litter (mg g^{-1} litter; release or uptake of the N to the organic N soil pool) by decomposition was calculated accordingly to the change in N content post field incubation:

$$Net\ N\ change = \frac{DW_{ini} \times \%N_{ini} - DW_{final} \times \%N_{final}}{DW_{ini}} \quad (2.12)$$

where: $DW_{ini/final}$ is the dry weight of the litter sample in g and $\%N_{ini/final}$ is the litter N content at start and end of incubation, respectively.

2.11 Estimation of the nitrogen balance of the Yatir forest and shrubland

2.11.1 Nitrogen balance pools and fluxes

In order to calculate aboveground (AG) and belowground (BG) biomass N, pools and fluxes of N in the two study ecosystems were quantified. We also combined our dataset of field measurements with previously published data from the same region. For field measurements we used three to five plots 30×30 m or 1 ha size in the central part of the forest and in surrounding shrubland. The shrubland plots were sub-divided into two microsites: under shrub canopy (shrub), and inter shrub interval (inter-shrub).

2.11.2 Aboveground biomass

AG biomass at the afforested sites was estimated from measurements of stem diameter at 130 cm and height of all the trees at five 30×30 m plots in the central part of forest using allometric equations (Grünzweig et al., 2007). AG biomass at the shrubland sites was obtained from Grünzweig et al., (2007), Kohanes and Sternberg

(personal communications), Sternberg and Shoshany, (2001) and from the report of Ariza, (2004).

AG litter layer biomass for both forest and shrubland was taken from Grünzweig et al., (2003) and Grünzweig et al., (2007). Litter flux in the forest was measured during years 2001-2005 by litter traps (25 litter traps of 0.5 m^2 size) at five plots inside the forest (5 traps per plot). For estimation of the shrubland litterfall we used averaged litterfall dry mass (dm) from the nearby Lehavim Long-Term Ecological Research station (Thompson et al., 2003; Thompson et al., 2006; 36 - 422 g litter m^{-2}), and the report of Osem et al., (2004) for the nearby Lahav area (100-200 g litter m^{-2}). Both sites (Lahav and Lehavim) situated in *S. spinosum* dominated shrubland within less than 10 km from our research site. The sites have similar precipitation, altitude, and soil properties.

The biomass of annuals in the forest was measured by sampling standing biomass in the forest plots in the 2001 - 2002 season. For estimation of the annual vegetation in the shrubland sites we used Grünzweig and Körner, (2001); Ariza, (2004) and Osem et al., (2004) reports, (120 g m^{-2}). For the annual scale N balance, we assumed that the annuals' biomass is fully recycled on a yearly basis.

2.11.3 Belowground biomass

BG biomass of live and dead root pools at the forest site were estimated by core sampling to 0 - 20 cm depth (5 plots and 5 replicates per plot) during 2006 - 2007 season. The root pool (i.e. dm of roots) for the shrubland was obtained from the review of Jackson et al., (1996), which reports mean value of 0.27 kg m^{-2} for roots dm in semi-arid environments. We combined this estimate with our measured (see below) root N concentration (1.17% N; mean value from bulk samples collected in the three shrubland plots), which yielded an estimate of 3.3 g N m^{-2}, similar to Bennett et al., (2003), for semi-arid grassland in Australia (3.5 g N m^{-2}).

Root mortality, decomposition, and production for the forest were calculated similarly to Matamala and Schlesinger, (2000), using the model of Santantonio and Grace, (1987). For model parameterization we used *in situ* measurements of wet and dry season decomposition rates (see below), and biomass of fine roots (dead and live) over three sampling dates in 2007 (January, March and June). Decomposition

coefficients (k) used in the model were 0.03±0.01 and 0.07±0.01 $month^{-1}$ for the dry and wet season, respectively. The calculated roots production rates were used to estimate below ground net primary productivity (BNPP) at the forest site. Alternative means of estimating BNPP, such as minirhizotron (Steinaker and Wilson, 2005) or ingrowth cores (Majdi et al., 2005) were not available to us, but the model could be partly validated by measured parameters. The model estimate of root decomposition was 21.5% mortality, which compared well with rates of root litter dm loss in field incubations (15.6±1.9%). The model calculated N release from decomposed litter (decomposition rate times root N%) also similar to measured values, 10% vs. 15.5±3.8%, respectively. For the shrubland sites, root litter production was estimated at 140 g m^{-2} based on mean values from Jackson et al., (1996) and Jackson et al., (1997). Root decomposition rates and N release during decomposition were measured by a litter decomposition assay (see Chapter 2.11).

2.11.4 Above-ground and below-ground growth requirements

AG growth requirements for the forest plots (2001 - 2004) were calculated based on litterfall measurements, and biomass (dm) increments for each tree appendage (i.e. brunches, twigs, cones, leaves, and stem), based on dendrobands and allometric equations developed specifically for the *P. halepensis* trees in Yatir forest (see Grünzweig et al., 2007). For shrubland, AG growth requirements were estimated from the increase in shrub biomass according to the increase in shrub land cover measured from aerial photographs of an undisturbed plot near the forest, obtained from Reisman-Berman, (2004) and Reisman-Berman et al., (2006), which yielded growth rate of 1.4% y^{-1}. Note that growth patterns of *S. spinosum* are characterized by fast growth upward during early stated (first ~5 y), and increase is spatial cover afterwards (up to 17 - 20 y; Litav and Orshan, 1971; Seligman and Henkin, 2003), indicating strong correlation between the land cover change and growth in the mature shrubland. In both ecosystem types, the N loss in leaf litterfall was assumed to be replaced on annual scale, and the amount of N loss in the litter was added into the estimated requirements.

For estimation of BG growth requirements in the forest site, we estimated fine root production based on the model of Santantonio and Grace, (1987), using measured decomposition rates (see 2.10) and live and dead root pools (see 2.11.3). At the

shrubland site, we assumed that all N loss by root litter production need to be replaced in addition to N required for the growth. BG biomass production was assumed to be similar to that of AG, i.e. 1.4% annually.

2.11.5 Soil pools and fluxes

The mineral soil inorganic N pool was measured by mineral soil sampling (50 mm core diameter, BenMedows Corp) to a depth of 20 cm at three plots (5 replicates per plot) during October 2005 at the forest and at the shrubland plots (for the shrubland plots the two microsites, *shrub* and *bare*, were sampled and the results were weighted by relative soil cover (see below). Total soil N pools for both ecosystems (0 - 20 cm, assumed to be mostly organic) were obtained from Grünzweig et al., (2007). Net N mineralization rates in soil and N deposition into the ecosystems were measured *in situ* (see Chapter 4), the wet N deposition to the ecosystems was corrected based on the amount of rainfall.

Biological Nitrogen Fixation (BNF) was estimated based by measurement of the biological soil crust (BSC) ground cover in five 1 ha plots, each, in the forest and shrubland sites. Within these plots 4 diagonal transects were marked and a BSC area was quantified (±2 cm^2) in three 0.1×0.1 m randomly selected sections within 0.4×0.4 m frames that were placed on free soil cover along the diagonals at ~1 m intervals. A total of 120-150 samples per plot were quantified and averaged for BSC cover.

The BSC cover was corrected for rock and stumps based on (Gelfand et al., submitted; 86±5% and 56±2% free surface in forest and shrubland, respectively) and yielded estimates of 26.7±2.3% and 44.7±2.7% cover in the forest and shrubland ecosystems. A range of N fixation rates by BSC was then estimated by applying the rates measured at the same region by Zaady et al., (1998; ~18 kg ha^{-1} y^{-1} using the acetylene method) and Russow et al., (2005; ~11 kg ha^{-1} y^{-1} using ^{15}N at the natural abundance level).

Gaseous emissions were estimated from extrapolations of laboratory results for nitric oxide emission (Chapter 5) and measurements of ammonia volatilization in the field. The nitrous oxide emission was assumed to be 5% of that of the nitric oxide (Conrad, 2002). Ammonia volatilization was measured by incubation of aliquot trapping solution (2% sulfuric acid (H_2SO_4) in deonized water) in the field. The acid

traps were placed in covered soil chambers (about 100 l volume, 0.6 m diameter) for about 8 h at four occasions during the wet and dry seasons of 2003 - 2004 at the forest site and assumed to be similar between the sites due to similar soil pH (Grünzweig et al., 2007; Chapter 4).

2.11.6 Nitrogen Use Efficiency calculation

The Nitrogen Use Efficiency (NUE) for above and below ground components of the ecosystems was calculated separately accordingly to the definitions of Berendse and Aerts, (1987) and Vitousek, (1982). The definition of Berendse and Aerts, (1987) expresses NUE (kg dw kg^{-1} N) as:

$$NUE = A \times \frac{1}{Ln} \tag{2.13}$$

where: A is N productivity term expressed as rate of below or above ground net primary productivity per unit of N in the above or below ground parts of the plants (kg dw kg^{-1} N y^{-1}):

$$A = \frac{NPP}{N_{biomass}} \tag{2.14}$$

Ln is N requirement for maintenance per unit of biomass; Ln is calculated as litterfall N per unit of N in the above or below ground plant biomass (kg N kg^{-1} N y^{-1}):

$$Ln = \frac{N_{litter}}{N_{biomass}} \tag{2.15}$$

Aboveground net primary productivity (ANPP) for the forest was taken from (Maseyk et al., submitted) and for shrubland (shrubs only) calculated as shrub biomass multiplied by the growth rate plus litter replacement. The below ground productivity (BNPP) for the forest was taken to be production rate of fine roots (see above), and for shrubland this was calculated as root biomass multiplied by the growth rate, plus litter replacement (similar to estimates of the N requirements, see above).

The second method for estimating NUE based on Vitousek, (1982) expresses NUE (kg dw kg^{-1} N litter) as:

$$NUE = \frac{dw_{litter}}{N_{litter}} \tag{2.16}$$

where: dw_{litter} is litter dry weight (kg) and N_{litter} is N concentration in litter (kg N kg^{-1}). Vitousek, (1982) showed that NUE calculated from this definition decreased with increasing nitrogen availability and therefore reflects ecosystem N limitation, which is widely used in ecological research (Moro et al., 1996; Fassnacht and Gower, 1999).

In order to estimate N availability from the trees internal pools were calculated, as N re-allocation from leaves prior to defoliation for the forest site by:

$$N_{reallocation} = (\%N_{leaf} \times M_{litter}) - (\%N_{litter} \times M_{litter}) \tag{2.17}$$

where: $\%N_{leaf}$ and $\%N_{litter}$ are mean %N in litter and green leaves for years 2001-2005, and M_{litter} is mean leaves litter mass for the same years.

2.12 Data processing and statistical analysis

All results on the N balance given in kg N ha^{-1} or kg N ha^{-1} y^{-1}. The results were upscaled according to soil cover, plant density, and soil properties. Statistical analysis of the results, regression analysis, student *t*-tests, and One-Way ANOVAs were obtained using the built in statistical functions of Origin 7.5, graphing and analysis software (OriginLab Corp., MA, USA).

Chapter 3

3 Nitrogen balance of the Yatir forest and the surrounding shrubland

3.1 Results

We present the measured and estimated components of the biogeochemical N cycle in the semi-arid Yatir forest and the surrounding shrubland ecosystems. The key components of the N balance in the semi-arid afforestation and surrounding shrubland are presented in Table 3.1. The major pools and fluxes are diagrammatically summarized in Figure 3.1 (a, b). Below we present the individual components.

3.1.1 Input

Overall N input by deposition was significantly higher in the forest than in the shrubland, but with high inter-annual variability. Annual values in the forest were 6.7±1.1 and 12.4±3.1 kg ha^{-1} for the 2004 - 2005 and 2005 - 2006 years, whereas the N input by deposition into the shrubland was 5.0±0.7 and 4.1±1.2 kg ha^{-1} for the same years. The bulk deposition (i.e., wet and dry deposition during the wet season) was significantly higher to the forest than to the shrubland, whereas the dry deposition (mostly dust during the dry season) did not differ significantly (Chapter 4). Inputs from BNF, whose estimate was based on the measured areas of bio-crust in the two ecosystems and the literature values (see Chapter 2), indicated higher input to the shrubland than to the forested area: 3.1 - 5.6 and 7.9 - 14.4 kg ha^{-1} y^{-1} for the forest and shrubland, respectively (Fig. 3.1).

3.1.2 Output

Nitrogen outputs may be due mostly to loss by volatilization and through dissolution and water loss to runoff and recharge. The only significant N loss in our ecosystems was estimated to be through runoff in the shrubland. Runoff in this ecosystem is about 3.2% (2001 - 2007 average; Laronne et al., 2007) of the annual precipitation (224 mm and 373.5 mm for 2005 and 2006, respectively). The loss

associated with this runoff is due to dissolved inorganic N (DIN) in rainwater and possible leaching from the soil. The DIN concentration in rainwater was measured, whereas the leaching was estimated based on literature values (Zaady, 2005), leading to an estimated total loss of between 0.1 and 1.5 kg ha^{-1} y^{-1}. Runoff in the forest was virtually zero (Laronne et al., 2007). Recharge to ground water in our region was recently estimated to be negligible (Shachanovich et al., 2007).

The NO emissions were estimated to be 0.2 and 0.24 kg ha^{-1} y^{-1} for forest and shrubland sites, respectively (Gelfand et al., submitted; Fig. 3.1). This indicates 16.7% lower NO emission in the forest than in the shrubland. The NH_4^+ volatilization rates, measured in the forest area and estimated in the shrubland area, were based on the literature values (Zaady, 2005). N_2O emission was estimated as 5% (Conrad, 2002) of the NO emission estimates. Both the NH_4^+ and N_2O emissions were considered negligible in our environment and were ignored in ecosystem budget calculations.

3.1.3 Main pools

The largest store of N was in the soil organic matter (0 - 20 cm layer) in both ecosystems: 1800±619 and 2075±648.5 kg ha^{-1} for shrubland and forest, respectively, which was not significantly different ($p = 0.11$). The N storage in the plant tissues (above and below ground) was significantly larger in the forest ($p = 0.05$) and was 131.5±28.0 kg ha^{-1} compared with 57.4±3.3 kg ha^{-1} in the shrubland. The litter layer in the forest accumulated 25.4±4 kg ha^{-1} of N, 64.6% more than litter layer in the shrubland, which accumulated only 9.0±3.0 kg ha^{-1} of N. Our estimates of the dead fine roots N pool indicated similar values, 16.8±0.9 and 16.4 kg ha^{-1} for forest and shrubland, respectively. The inorganic N pool in the mineral soil at the end of the dry season was larger in the forest than in the shrubland, 4.9 - 5.1 kg ha^{-1}, and 1.7 - 4.1 kg ha^{-1}, respectively (2005 - 2006; Fig. 3.1).

3.1.4 Main fluxes

The leaf litterfall in the afforestation contained less N than the litterfall in the shrubland, with 9.0±0.2 and 11.9 kg for the two ecosystems, respectively. The dry weight of the litterfall biomass was greater in the forest than in the shrubland, 1878.8±36.4, and ~1000 kg ha^{-1} y^{-1}, respectively (measured for forest and estimated for

shrubland, see 2.11.2). The lower N concentration in the pine litter and the higher total N concentration in the pine above-ground biomass was reflected in the slower turnover rate of N in the forest (Table 3.1). The N flux to the dead roots pool, calculated as root mortality (based on Santantonio and Grace, (1987)) was slightly lower in forest, 14.6±2.1 vs. 16.4 kg ha^{-1} y^{-1} for forest and shrubland, respectively (Fig. 3.1). The decomposition rate of the leaf litter in the forest, expressed as dm loss, was slower, on average, than the rate of decomposition in the shrubland, but these differences were not significant (Fig. 3.2). The root decomposition rate, expressed as dm loss, was significantly lower in the forest than in the shrubland (Fig. 3.2).

During the *in situ* annual litter decomposition incubation measurements, pine leaf litter exhibited an increase in N concentration of 0.45±0.07 mg g^{-1}, whereas the litter incubated in shrubland exhibited a net decrease in N concentration of 5.00±0.34 mg g^{-1}. The roots litter exhibited a net N concentration decrease of 1.06±0.13 and 4.20±0.52 mg g^{-1} in the forest and the shrubland, respectively (Fig. 3.3). Overall, the annual decomposition added 2.5±0.4 and 10.9±2.1 kg ha^{-1} y^{-1} to the total soil N pool in the forest and in the shrubland, respectively (Fig. 3.1). The average net N mineralization rate in shrubland was higher than that in the forest: 16.4±9.1 and 30.8±16.5 kg ha^{-1} y^{-1}, respectively, but these differences were not significant (Fig. 3.4; $p = 0.13$),

The N growth requirements for the *P. halepensis* trees were calculated (AG and BG combined) to be 42.2±14.2 kg ha^{-1} y^{-1}, which was not significantly different from the estimated N requirements of *S. spinosum* of 28.8 kg ha^{-1} y^{-1}.

N contained in the biomass of annual plants was assumed to be fully recycled on an annual basis. Growth of annuals was slightly reduced in the afforestation system with recycling of 12.1±3.7 and 18.2±1.8 kg N ha^{-1} y^{-1} for forest and shrubland sites, respectively (Fig. 3.1).

3.1.5 Nitrogen Use Efficiency

The nitrogen use efficiency was higher in the forest than in shrubland, for both AG and BG plant components (Table 3.2). This was the case when NUE was calculated either according to Vitousek, (1982), which yielded 208.8±6.6 vs. 84.0 kg dw kg^{-1} N (for AG efficiency) or according to Berendse and Aerts (1987), which

indicated 430.1 vs. 87.7 kg dw kg^{-1} N, for AG biomass and 329.0 vs. 87.6 kg dw kg^{-1} N, for BG biomass in the forest and shrubland, respectively. The high NUE in the forest also reflected longer N MRT in the pine trees, in comparison with the shrubs (8.4 vs. 2.1 y^{-1} for AG and 3.9 vs. 2.0 y^{-1} for BG). The N productivity of the pine trees was also higher than that of the shrubland (48.4 vs. 41.8 for AG and 85.5 vs. 43.8 for BG in the forest and shrubland, respectively; Table 3.2). NPP in the forest and shrubland was estimated to be 3653.0 vs. 1024.0 for AG and 4785.7 vs. 1437.8 for BG in the forest and shrubland, respectively (Table 3.2).

3.2 Discussion

A large increase in carbon storage, associated with afforestation of semi-arid shrubland, was not associated with a significant change in the soil N stock (Grünzweig et al., 2003; Grünzweig et al., 2007). This raised questions regarding the factors that often control the carbon cycling in the terrestrial biomes, such as water and N availability. Results of a two-year irrigation experiment conducted in the Yatir forest indicated a further large increase (~34%) in final needle length (Maseyk, 2006). Thus, adding water during the dry summer significantly increased biomass production, indicating no obvious N limitation. This further suggested an increase in NUE that partly motivated the present study. However, because of the natural N content of the irrigation water, this treatment could introduce significant amounts of N (3 mg N l^{-1} translating to ~20 kg ha^{-1} y^{-1}). Since this is approximately two times larger than the annual N deposition into the ecosystem, it precludes clear conclusions regarding the predominance of water or the N limitation on biomass production in the afforestation system. In the present work, we show that there was no significant additional N deposition or any other external N sources to the forest. However, there were clear adjustments in the ecosystem N cycle associated with changes in litter quality, the rates of decomposition and mineralization, NUE, N turnover, and N residence time. These changes clearly help explain the large biomass increase (and carbon sequestration) associated with afforestation of semi-arid shrubland.

The N requirements of the *P. halepensis* trees were calculated to be greater than the requirements of the *S. spinosum* shrub (Table 3.1). The greater N requirements of the *P. halepensis* were reflected in a nearly balanced N budget for the ecosystem, in

contrast to the clearly positive net N balance in the shrubland (Table 3.1). A negative N balance for forests is not uncommon (Chestnut et al., 1999, and references therein), but the imbalance in the input/output fluxes is within the uncertainties of the present study: It may arise from re-allocation of N from the leaves before defoliation (46.5% of the average leaf N in green leaves or 8.0±0.2 kg ha^{-1} y^{-1}), which could explain the difference between forest N requirements for growth and N available (-3.3 to 5.0 kg ha^{-1} y^{-1}; Table 3.1). Significant N re-allocation from senescing leaves is consistent with the low litter quality in our system. Additionally, throughfall N and gaseous N uptake by the leaves can contribute as much as ~4 kg ha^{-1} y^{-1} (Moro et al., 1996) but this was not estimated here. Our estimation of BNF could result in underestimating this component, since it was based on the soil cover measurements combined with rates from other studies. Finally, using net N mineralization rather than gross N mineralization rates (Davidson et al., 1991; Knops et al., 2002) may also result in underestimation of available N from the mineralization process.

The N stored in the plant tissues (AG and BG) was significantly larger in the forest than in the shrubland (131.5±28.0, and 57.4±3.3 kg ha^{-1} for the forest and shrubland, respectively). However, the N loss by litterfall was smaller (23.6±3.9 and 28.3 kg ha^{-1}) in the forest than in the shrubland, respectively (Fig. 3.1). Together with increased carbon and N productivity and a significantly larger C/N ratio in the forest (Grünzweig et al., 2007), these changes result in an almost fivefold higher aboveground NUE in the forest than in the shrubland (406.4 vs. 87.8 kg dw kg^{-1} N, Table 3.2). This was associated with a fourfold increase in N MRT in the forest AG biomass, and more than a 1.5-fold increase for BG biomass (Table 3.2). These changes in the NUE and N MRT and N productivity are probably key elements in explaining the large increase in the above-ground organic C stock, >25-fold, over the 35 years afforestation (Grünzweig et al., 2007). The NUE of the forest and surrounding shrubland, calculated as the ratio between the dm loss in litterfall and the N returned to the soil by litterfall (Vitousek, 1982), was in the range of NUE values reported for other Mediterranean and temperate areas of the globe (Moro et al., 1996; Fassnacht and Gower, 1999).

Belowground NUE is not often calculated due to methodological difficulties in measuring belowground processes, but here we show that its values were also higher in

the forest than in the shrubland (329.0 vs. 87.6 kg dw kg^{-1} N; Table 3.2). This is consistent with the higher BG N productivity in the forest stand, 85.5 vs. 43.8 kg dw kg^{-1} N y^{-1} in the shrubland, and the high belowground NUE and longer N MRT in this ecosystem (Table 3.2).

Comparison of specific N fluxes in the two studied ecosystems demonstrates the consequences of afforestation on the ecosystem N balance. The bulk deposition increased in the forest by 1.8 kg ha^{-1} y^{-1} on average (probably mainly owing to leaf dust collection and its washout), which was associated with decreased runoff and gaseous emissions (Fig. 3.1). The reduction in N loss in runoff is mainly due to increased tree cover combined with the presence of understory annuals during the rainy period. Despite the fact that local runoff is possible in the Yatir forest, the runoff at our research site was found to be undetectable (Shachanovich et al., 2007). In the surrounding shrubland the runoff/precipitation ratio was found to be on average 3.2% (but much higher during high intensity rain events; Laronne et al., 2007).

In contrast with the decrease in the N output through runoff, we estimated that the afforestation also resulted in reduction of N input through BNF (Fig. 3.1). This might be attributed to the inherently slow recovery rate of the soil biological crust after disturbance by the afforestation process (Evans and Belnap, 1999), or to slow recovery due to the allelopathy effect of pine leaf litter (Paavolainen, 1999). The combined effects of reduced N loss (runoff), reduced N input (biocrust), and a small N increase in bulk deposition help explain the overall nearly equal N inputs to both ecosystems (Table 3.1).

In contrast to the lack of significant changes in the net N balance of the two ecosystems, there were other internal changes that help support the increased productivity of the forest, in particular, changes associated with the wet, productive season. For example, the additional N input by the bulk deposition, although not significantly influencing the annual budget, was associated with rainfall in the productive season, the additional soil inorganic N pool at the beginning of the wet season (Fig. 3.1), better synchronization between plants' N demand and N availability (Chapter 4), and re-allocation of N from senescing leaves (Maseyk, 2006; this study).

In addition, the forest was relatively “conservative” in its N cycling. For example, litter decomposition, N release from the litter, and mineralization were

slower in the forest, similar to other evergreen forests (Waring and Schlesinger, 1985). N immobilization into the litter, observed during the first stages of decomposition in the forest (Fig. 3.2; Fig. 3.3), is also a common phenomenon in pine forests (Garcia-Pausas et al., 2004; Berg and Laskowski, 2005b). The slower decomposition rates of the litter may be associated with the lower litter quality, which had a much higher C/N ratio (Torres et al., 2005).

Despite slower decomposition rates of forest litter, the N flux to the litter pool should be balanced by N flux from the litter to the soil pool, unless there is a continuous increase in litter pool. The latter is unlikely, under (quasi-) steady-state assumption (Brumme and Khanna, 2008). Moreover, earlier studies on the Yatir forest indicated that there was no development of the litter layer (Grünzweig et al., 2003; Grünzweig et al., 2007). Such assumptions allow estimating the previous year's decomposition or mycorrhizal N additions to the organic N soil pool, which were 22.2 and 17.4 kg ha^{-1} y^{-1} for the forest and shrubland, respectively.

In addition to slower decomposition rates, the forest soils exhibited reduced net N mineralization rates, which possibly account for the increase in N_{org} pool in the SOM of the forest (Fig. 3.1). The differences between total N pools and the N mineralization rates of the shrubland and forest ecosystems were not statistically significant ($p = 0.11$ for the soil total N pools Fig. 3.1; $p = 0.13$ for net N mineralization). The slower net N mineralization in the forest was in the range of published rates (Davidson et al., 1991; Gallardo and Merino, 1998; Bustamante et al., 2006; Singh and Kashyap, 2006). These changes in the internal N cycling represent the tighter and slower N cycling in the forest in comparison with the shrubland.

In summary, the increase in the NUE, together with increased focusing of available N during the active season, as well as possible synchronization between N availability and N demand for photosynthetic activity help explain the relatively large increase in biomass and carbon sequestration associated with pine afforestaiton in this semi-arid forest.

Table 3.1 Main compartments related to the N balance of the Yatir forest and shrubland ecosystems. The net external N balance is calculated as the difference between external input and output from the ecosystem. Turnover rate, *Ln*, was calculated as $Ln = \frac{N_{litter}}{N_{biomass}}$ (kg N kg^{-1} N y^{-1}). Requirements estimated as the sum of aboveground and belowground compartments of N demand for growth. Net N balance was calculated as the sum of all annually available N (external balance + soil inorganic N + N mineralization + N relocation - growth requirements).

	Yatir forest	Shrubland
Total Input (kg N ha^{-1} y^{-1})	9.8 - 18.0	12.0 - 19.4
Total Output (kg N ha^{-1} y^{-1})*	0.2	0.3 - 1.7
Net external balance	9.6 - 17.8	11.7 - 17.7
Soil Inorganic N (kg N ha^{-1})**	4.9 - 5.0	1.7 - 4.1
N mineralized from soil organic N (kg N ha^{-1} y^{-1})	16.4 (9.1)	30.8 (16.5)
Requirements (kg N ha^{-1}y^{-1})***	42.2 (14.2)	28.8
N relocation (kg ha^{-1} y^{-1})	8.0 (0.2)	-
Net N balance	-3.3 - 5.0	15.4 - 23.8
Turnover rate (*Ln*) (y^{-1})****	0.12 / 0.26	0.48 / 0.50

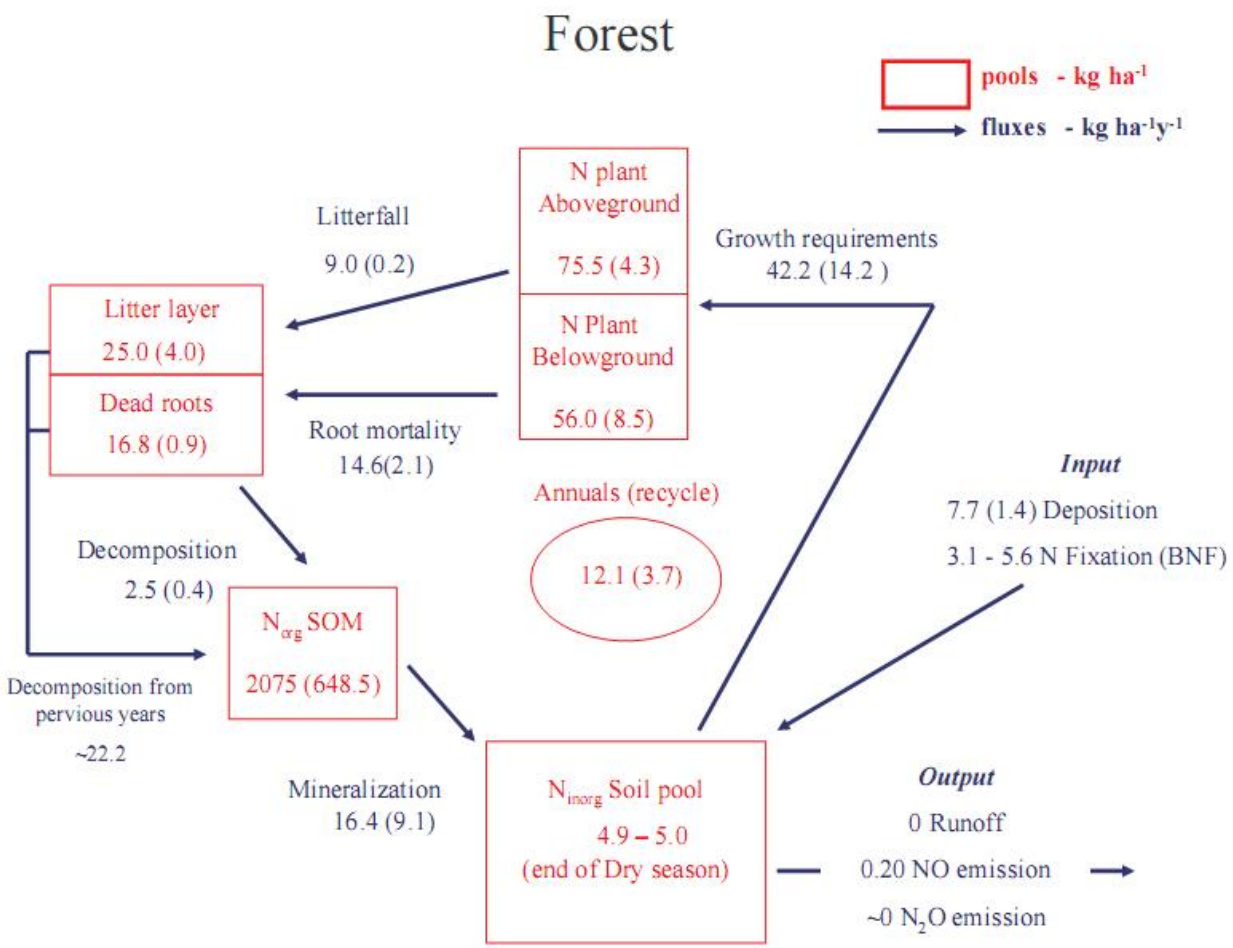
Each value is the mean (±SE), when available.
* Growth requirements are not included; ** Inorganic N pool at the end of the dry season with a 0-20 cm soil layer; *** Aboveground + Belowground; **** Aboveground / Belowground

Table 3.2 Nitrogen use efficiency (NUE) components in the Yatir forest and shrubland. The NUE (kg dw kg^{-1} N) was calculated according to (I) Berendse and Aerts, (1987) and (II) Vitousek, (1982). NPP = net primary productivity (kg dw ha^{-1} y^{-1}); A = N productivity (kg dw kg^{-1} N y^{-1}); mean residence time of N in the ecosystem (MRT) = 1/*Ln* (y).

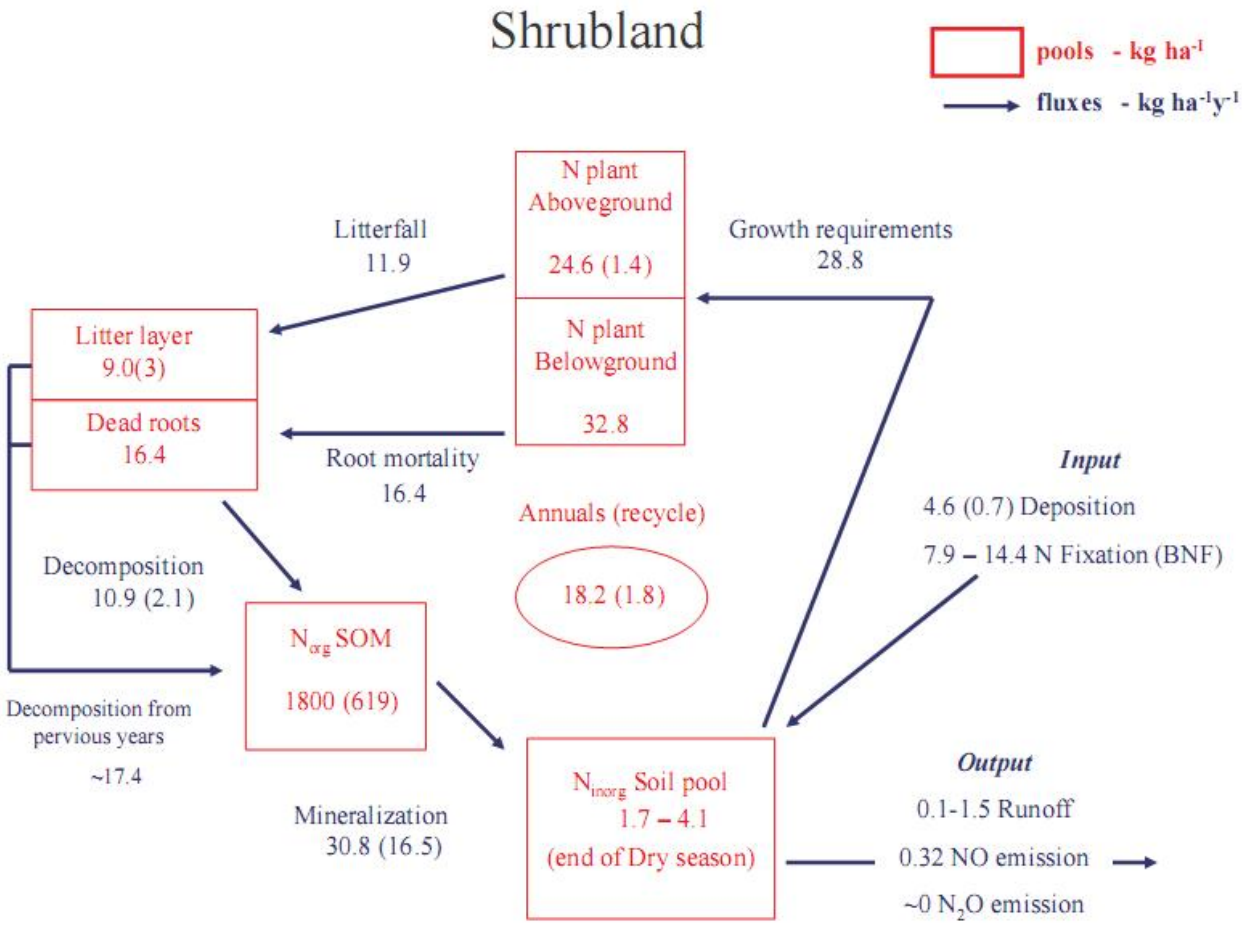
	Yatir forest		Shrubland	
	Aboveground	Belowground	Aboveground	Belowground
NPP (kg dw $ha^{-1}y^{-1}$)	3653	4785.7	1027	1437.8
A (kg dw kg^{-1} N y^{-1})	48.4	85.5	41.8	43.8
MRT (y)	406.4	3.9	2.1	2.0
NUE (I) (kg dw kg^{-1}N)	430.1	329.0	87.8	87.6
NUE (II) (kg dw kg^{-1}N)	208.8±6.6	-	84.0	-

Table 3.3 Dry (total N) and Bulk (inorganic N) deposition (kg N ha^{-1} y^{-1}) in the Yatir forest and surrounding shrubland. Significant differences between values are shown by letters ($p<0.05$); Values are means ±SE ($n = 3$)

	Dry deposition		Bulk deposition	
	Forest	Shrubland	Forest	Shrubland
2004-2005	0.8 ± 0.4^{a}	0.9 ± 0.3^{a}	6.7 ± 1.1^{a}	5.0 ± 0.7^{b}
2005-2006	0.7 ± 0.3^{a}	1.3 ± 0.8^{a}	12.4 ± 3.3^{c}	4.1 ± 1.2^{d}


Forest
pools - kg ha-1
fluxes - kg ha-1y-1
N plant Aboveground
75.5 (4.3)
N Plant Belowground
56.0 (8.5)
Litterfall
9.0 (0.2)
Growth requirements
42.2 (14.2)
Litter layer
25.0 (4.0)
Dead roots
16.8 (0.9)
Root mortality
14.6(2.1)
Annuals (recycle)
12.1 (3.7)
Input
7.7 (1.4) Deposition
3.1 - 5.6 N Fixation (BNF)
Decomposition
2.5 (0.4)
Norg SOM
2075 (648.5)
Decomposition from pervious years
~22.2
Mineralization
16.4 (9.1)
Ninorg Soil pool
4.9 – 5.0
(end of Dry season)
Output
0 Runoff
0.20 NO emission
~0 N2O emission

a



b

Figure 3.1 The nitrogen balance of the Yatir afforestation (a, p. 42), and surrounding shrubland (b, p. 43). Standard error values are given in parentheses when suitable; numbers without errors are calculated or estimated based on literature reports.

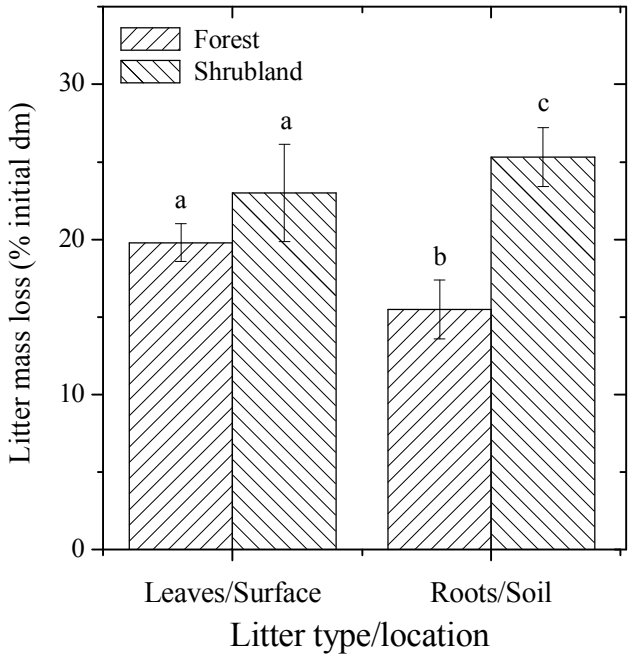


Figure 3.2 Mass loss of local litter at forest and shrubland sites during the annual incubation. The letters indicate the significant differences between treatments ($p<0.05$). Values are mean ±SE, n = 4-5 plots.

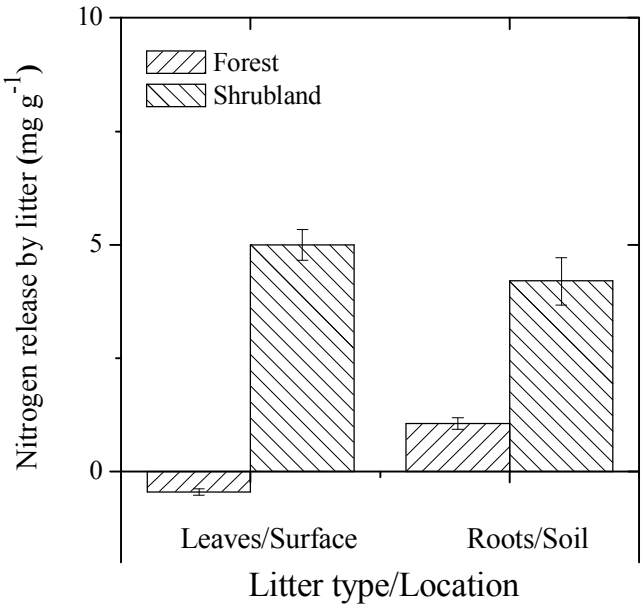


Figure 3.3 Change in nitrogen concentration of litter at the forest and shrubland sites during annual incubation. Values are mean ±SE, *n* = 4-5 plots (2 - 5 samples per plot, bulked)

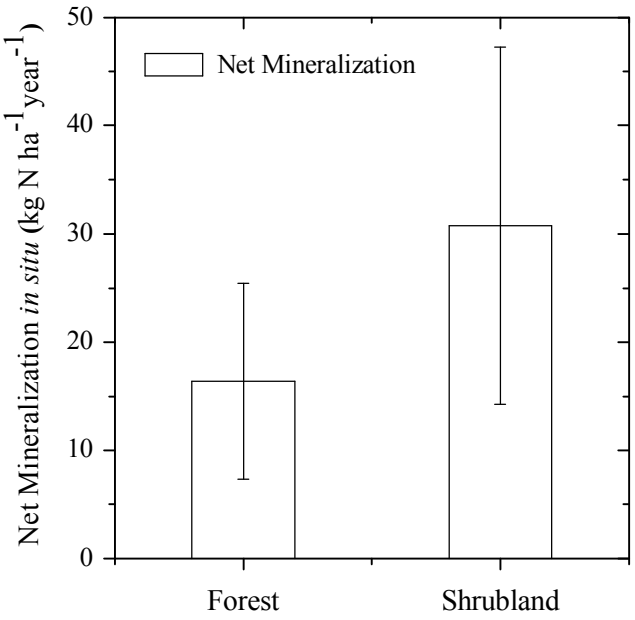


Figure 3.4 Net N-mineralization *in situ* (kg N ha^{-1} year^{-1}) during the 2004 - 2005 season at forest and shrubland sites. Mean ±SE, n = 3 plots.

Chapter 4

4 Nitrite accumulation in association with seasonal patterns and mineralization of soil nitrogen in a semi-arid pine forest

4.1 Results

4.1.1 Nitrogen deposition

Dry deposition N input to the ecosystems did not differ significantly between afforested sites and shrubland sites during both seasons (2004 - 2005 and 2005 - 2006). In contrast, bulk deposition was significantly different during both seasons (Table 3.1). Testing for possible additional nitrite originating from rainfall indicated that this source was insignificant (Table 4.1). Although there was a clear increase in nitrite concentrations in the mineral soil, this could not be explained by the bulk deposition.

4.1.2 Seasonal changes of inorganic N concentration in the mineral soil

The 0-10 cm mineral soil layer of afforested plots displayed seasonal variation in inorganic N (Fig. 4.1a). The $N\text{-}NH_4^+$ concentration varied from 0.3±0.2 µg N g^{-1} sdw during the wet season (February-May) up to 3.1±0.4 µg N g^{-1} sdw at the end of the dry season (May-October). $N\text{-}NO_2^-$ concentration showed little variation and the average values were from below the detection limit, up to 0.3±0.0 µg N g^{-1}sdw over the dry season. However, after rain and an increase in soil water content, nitrite concentration in the soil increased up to 1.7±0.9 µg N g^{-1} sdw, whereas ammonium concentration decreased. $N\text{-}NO_3^-$ levels were low, ranging from below the detection limit during the dry season up to 0.8±0.3 µg N g^{-1} sdw during the wet season. At the shrubland plots, $N\text{-}NH_4^+$ concentrations were similar to those in the afforested plots, but the increase in $N\text{-}NO_2^-$ concentrations was much less pronounced (up to 1.1±0.5 µg N g^{-1}sdw), and the $N\text{-}NO_3^-$ concentrations increased up to values as high as 4.2 µg N g^{-1}sdw (Fig. 4.1 b, c).

4.1.3 Mineralization *in situ*

Mineralization *in situ* exhibited a seasonal pattern in all plots, with significant differences in rates between seasons and sites (Fig. 4.2). Mineralization rates were low in the dry period (June - October); inorganic nitrogen removal from cores (immobilization by soil microbes) was observed in the rewetting period (October - December), and mineralization rates were high in the wet period (December - May; Fig. 4.2). Considering the differences among sites, note that although we separated the wet season into two sub-periods, the general patterns displayed similarity in mineralization rates between the *forest,* and *bare* soils, but the rates in the *shrub* sub-plots were higher. Low activity with no differences among sites was observed in the dry season, and significantly different intensities of reactivation (reflected in immobilization) were observed in the re-wetting period (Fig. 4.2).

4.1.4 Laboratory incubations

In slurry incubations, nitrite started to accumulate in the initial hours of the incubation and continued to accumulate with relatively constant accumulation rates until the end of the experiments. Nitrite accumulation rates were 0.10, 0.07, and 0.13 μg N g^{-1} sdw h^{-1} in October, December, and February, respectively (Fig. 4.3). During October the incubations of soils from the shrubland showed a similar trend, with different nitrite accumulation rates: 0.01 and 0.11 μg N g^{-1} sdw h^{-1} for soils from the *shrub* canopy microsite and 0.02 and 0.05 μg N g^{-1} sdw h^{-1} for soils from the *bare* microsite, in October and February, respectively (Fig. 4.4).

Ammonia oxidation started immediately upon incubation and the rates were similar in incubations of soil samples from different months; they were 0.08, 0.10, and 0.10 μg N g^{-1} sdw h^{-1} for October, December, and February, respectively. The incubations with shrubland soils showed similar trends and rates; incubations with soils from the *shrub* microsites had ammonia oxidation rates of 0.18 and 0.17 μg N g^{-1} sdw h^{-1} and those from the *bare* microsites 0.15 and 0.07 μg N g^{-1} sdw h^{-1} for October and February, respectively (Fig. 4.4).

In contrast to nitrite, soil slurries from the *forest* from the end of the dry season (October) exhibited nitrate accumulation with a delay of about ~50 hours from the beginning of the incubation (Fig. 4.3a). Soils from the shrubland exhibited low

accumulation rates of 0.05 and 0.07 μg N g^{-1} sdw h^{-1} for soils from the *shrub* canopy and *bare* microsites, respectively (Fig. 4.4a, c). After the onset of nitrate accumulation, the accumulation rate in incubations with dry-period-soil (October) was 0.36 μg N g^{-1} sdw h^{-1} for *forest* soils, 0.47 μg N g^{-1} sdw h^{-1} for soils from *shrub* canopy microsites, and 0.52 μg N g^{-1} sdw h^{-1} for soils from the *bare* microsites (Fig. 4.3a; Fig. 4.4a, c). Incubations of soil from *forest* sampled at the rewetting period (December) displayed two different rates of nitrate accumulation: during the first ~40 hours, the nitrate accumulation rate was 0.07 μg N g^{-1} sdw h^{-1} and was equal to the rates of nitrite accumulation. However, after ~50 hours, nitrate accumulation rates increased to 0.39 μg N g^{-1} sdw h^{-1} and were close to rates observed in incubations with wet-period-soil (February; Figure 4.3b).

In contrast to dry-period-soils, in the wet-period-soil (February) nitrate started to accumulate immediately in experiments with all soil types, with rates of 0.30, 0.48, and 0.33 μg N g^{-1} sdw h^{-1} for *forest*, *shrub*, and *bare* microsite soils, respectively (Fig. 5.3c; Fig. 4.4b, d). The rates of nitrate accumulation in these samples were higher than those of nitrite during the entire incubation (Fig. 4.3c; Fig. 4.4b, d). In incubations without soil (with medium only) or with sterile soil, neither nitrite nor nitrate accumulation or ammonia oxidation was detected (Fig. 4.4e, f).

4.2 Discussion

Nitrogen availability is a key factor in ecosystem productivity and it is generally accepted that terrestrial ecosystems are often nitrogen limited (Vitousek and Howarth, 1991). We studied aspects of the nitrogen cycle that may have consequences for plant nitrogen availability in a semi-arid afforestation ecosystem. Specifically, we studied seasonal patterns of atmospheric N deposition, inorganic N concentration changes in the mineral soil, and mineralization rates in soils from the Yatir afforestation area and the surrounding shrubland for over two years. We conducted laboratory incubations of soil from the different seasons and we discuss mechanisms of nitrite accumulation in the mineral soil (0 - 10 cm depth).

4.2.1 Nitrogen deposition

Nitrogen input by dry deposition was similar in the afforested and shrubland plots, although the bulk deposition showed larger N input in the forested plots (Table 4.1). We attribute these differences to the additional N that originated from the dust washed from the branches of trees by rain. Calculated on a yearly basis, N deposition in the afforested plots increased by a small, but significant amount (1.8 kg N ha^{-1} y^{-1}; based on measured wet deposition between May 2004 - February 2006) compared with the shrubland. Considering that afforestation of the shrubland resulted in almost a threefold increase in the standing biomass over about 40 years (Grünzweig et al., 2003), the lack of a pronounced increase in N input in the afforestation ecosystem raises questions about the N dynamics in the soil profile.

4.2.2 Seasonal N pattern

The seasonal pattern of inorganic nitrogen concentration in the soil followed that of water availability and plant activities. The dry season (June - October) showed very low activity of the afforestation ecosystem (low NEE; Fig. 4.1), including low mineralization rates in the soils (Fig. 4.2), associated with increased ammonium concentration. We generally assume that this low activity is dictated by the low soil water content during the dry season. Relatively high ammonium concentrations (up to 3.1±0.4 μg N g^{-1} sdw) at the end of dry season were consistent with relatively low *in situ* mineralization rate measurements (0.06±0.02 μg N g^{-1} sdw $week^{-1}$) during the dry season, and dry deposition measurements. The mineralization process during the dry season may occur as a result of the higher water content of the soil during early morning hours, which was previously observed in our system (unpublished results; Fig. 4.2). Additionally, the mineralization process is known to be less affected by drought than by other microbial-driven soil processes (Reynolds et al., 1999; Smolander et al., 2005).

After the first rains, the decreased ammonium concentrations were associated with increased nitrite concentrations, but there was only a small increase in nitrate concentration in the *forest* soils (Fig. 4.1a). Estimation of newly added inorganic nitrogen by deposition associated with rain events could account for the small increase of nitrate concentrations, but could not explain the increase in nitrite concentrations (Table 4.2).

Decreased ammonium concentrations in the soil after soil rewetting most likely represented NH_4^+ oxidation and uptake by microorganisms, and possibly by plants. The net immobilization of nitrogen in the first months of the wet season (Fig. 4.2) must reflect re-activation of soil microorganisms after rewetting (Fierer and Schimel, 2002; Fierer et al., 2003).

Ecosystem activity (reflected in NEE, see Fig. 4.1) in the Yatir afforestation reaches a peak between February and April, and at this time inorganic N concentration in the soil reached its annual minimum (Fig. 4.1a). This is expected, as discussed, for example by (Binkley and Hart, 1989), since high plant demands normally reduce the soil N pool. Similarly, increased concentrations of inorganic N in the soil during the dry season are also influenced by low demands by the vegetation. The transition from one state to another and the associated dynamics in N concentrations depends on microbial activities and the rate of conversion to N forms available for plant uptake, i.e., the rate of mineralization (Fig. 4.2).

The relatively high ecosystem productivity in the Yatir afforestation system (2 - 3 tC ha^{-1} y^{-1}; (Grünzweig et al., 2003; and see www.carboeurope.org), combined with almost unchanged N input by atmospheric deposition, and more than doubling of the plant C/N ratio in going from the background shrubland to the pine forest (see section 2.10), imply increased nitrogen use efficiency (NUE) of the afforestation (see Berendse and Aerts, (1987)). The implied increase in NUE most likely required some ecosystem adjustments related to soil N. We hypothesize that the peak in N concentration at the start of the wet season, together with the apparent delay in nitrate production, may provide better synchronization between N availability and the seasonal cycle in plant carbon assimilation (reflected in NEE) that peaks toward the end of the wet season. This can play a significant role in the relatively high productivity of this afforestation system (Grünzweig et al., 2003).

Overall, inter-annual variability in the patterns of changes in net ecosystem CO_2 exchange (NEE) seems to be influenced by the inter-annual variations in the seasonal patterns of precipitation, and in particular, the timing and intensity of the first rain events (Fig. 4.1). The inter-annual variability in concentrations of inorganic N in soils was also high, especially for nitrite, between the 2004 - 2005 and 2005 - 2006 annual cycles (Fig. 4.1b, c). Here too, we related this inter-annual variability to the different

precipitation patterns in the two annual cycles. Whereas in the first cycle, the wet season started with a significant rain event, the second annual cycle started with a small and sporadic rain event. This resulted in gradual re-wetting of the 0 - 10 cm layer, and most likely allowed better adaptation of the two types of microbial populations and, in turn, resulted in a less pronounced seasonal nitrite peak (Fig. 4.1 upper panel).

4.2.3 Nitrite accumulation in the mineral soil layer

In soils subjected to drought lasting up to 8 months under high temperatures (up to 62°C in surface soil, temperature measured at 2 cm depth), we can assume that most bacteria in the upper soil layer were inactive during the dry period. We hypothesize that since AOB populations are more tolerant to drought and to high temperatures than NOB populations, and since there is a faster recovery rate of AOB during rewetting, these factors determined the actual rates of ammonium and nitrite oxidations in such a ecosystem. Recently, Avrahami and Bohannan, (2007) found high potential nitrification activity under high temperatures (30°C) whereas the abundance of the ammonia oxidizing bacteria was very low. A possible explanation for such high activity is that AOB adapted to the stress conditions where the relatively low competitiveness of the ammonia oxidizing microorganisms is overcome by the fast initiation of activity. Decreasing the lag time may provide an advantage in nutrients-use efficiency compared to other soil microorganisms.

Tappe et al., (1999) found in recovery experiments with *N. europaea* (AOB) and *N. winogradskyi* (NOB) that the former was able to use ammonium immediately, whereas the latter needed up to a threefold longer period before it could start using nitrite. These findings, together with the finding that *N. europaea* competes better for limiting amounts of oxygen than *N. winogradskyi* (Laanbroek and Gerards, 1993), support our interpretation of the annual nitrite accumulation observed in the semi-arid ecosystem. Nejidat, (2005) recently found nitrite concentrations of up to 1918 μg g^{-1} of NO_2^- in soil samples from the Negev desert (under *Tamarix* trees), providing evidence for the occurrence of nitrite accumulation under natural field conditions in an arid ecosystem. In incubations of nitrifier microorganisms enrichment from those samples, high nitrite accumulation after a few days of incubation was observed, followed by subsequent oxidation of nitrite to nitrate. Nejidat, (2005) associated the accumulation of

nitrite during incubations of enriched soil with inhibition of NOB owing to high salt concentrations.

The *in situ* net N mineralization rates showed similar patterns of seasonal behavior in the different ecosystem types (i.e., afforestation and shrubland), although with differing magnitudes (Fig. 4.2). We assume that other microbial-driven processes of the N cycle (besides mineralization) may also have the same seasonal patterns and that the seasonal behavior of the soil microbial activity did not change by tree planting.

4.2.4 Nitrite accumulation in lab experiments

Nitrite accumulation in laboratory incubations and in the field can be explained by differences in rates between the first and the second steps of the nitrification process:

$$NH_4^+ \xrightarrow{k_1} NO_2^- \xrightarrow{k_2} NO_3^- \qquad 4.1$$

where k_1 is the rate of ammonia oxidation and k_2 is the rate of nitrite oxidation during nitrification, and NO_2^- accumulation can be, as observed here, interpreted as $k_1 > k_2$. This interpretation assumes that in short laboratory incubations, under optimized conditions, the microbial activity reflect the physiological state and maximized activity of these microbes *in situ*, in the environment. We noted, however, that the response time-scale in the laboratory incubation cannot be simply extrapolated to the field (e.g., a few hours delay in process activation in the lab, as discussed here, may as well mean a delay of weeks under field conditions).

Patterns in nitrification activity (e.g., ammonia oxidation, nitrite, and nitrate accumulation) in the laboratory incubations can be summarized by two main findings. First, ammonia oxidation rates were similar to nitrite accumulation rates in all experiments. Furthermore, both ammonia oxidation and nitrite accumulation proceeded immediately upon adding water (Fig. 4.3a). Second, once initiated, nitrate accumulation proceeded at similar rates in all soils from different seasons. The main difference between incubations of soils from different seasons was in the delay between the beginning of the experiment and beginning of the nitrate accumulation. Note that the similarity in the final oxidation rates from different periods may indicate stable microbial populations throughout the seasonal cycle.

In the laboratory incubations, we identified three main phases of soil nitrification activities. First, in incubations of end of the dry season soils (October), we essentially reproduced the soil-rewetting stage, in which the nitrifying populations begin to reactivate (Fig. 4.3a). Second, in incubations of soils from the beginning of the wet season (December), we reproduced an intermediate stage where all nitrifying populations are at least partly active (Fig. 4.3b). Third, in incubations of soils from the wet season (February), we reproduced a stage when all nitrifying populations are fully activated (Fig. 4.3c).

The AOB populations are known to be more drought-tolerant than NOB (Hastings et al., 2000) and would be expected to have an advantage under drought conditions, and $k_1 > k_2$ (Eq. 4.1). This should be reflected in nitrite accumulation in the medium in the first set of incubations (Fig. 4.3a).

The second incubations (Fig. 4.3b) most likely reflected the partial re-activation of nitrifying microbial populations in soil, and indeed the estimated rates of nitrite and nitrate accumulations are similar, and conditions for $k_1 = k_2$ probably existed (Eq. 4.1) during the first hours of incubation (Fig. 4.3b). This corresponded to an intermediate situation were AOB are fully activated whereas NOB are only partially recovered, and its activity is still restricted. At this stage, no significant NO_2^- accumulation in the field would be expected.

After full recovery of both AOB and NOB populations, the system probably reaches a short-term equilibrium for the wet period. The February soil slurry incubations were consistent with such conditions (Fig. 4.3c). The accumulation rate of nitrate was much higher than that of nitrite during the incubation, implying $k_1 < k_2$ (Eq. 4.1), and indicating that under these conditions there should be no or low nitrite accumulation in the field, which is associated with near zero nitrite concentrations in the soil, consistent with our observations (Fig.4.1a).

The idea that the patterns discussed above for the *forest* sites reflect some ecosystem-scale adjustments associated with the afforestation in this region is consistent with the observations of lower rates of nitrite and nitrate production (soil slurry incubations in the dry season) and lower levels of seasonal nitrite accumulation in the shrubland (Fig. 4.4).

Table 4.1 Soil inorganic nitrogen content (µg N g^{-1} sdw) at the end of the dry season and after the first rain events at the forest plots (rain events of start of 2004-2005 wet season, October-December, 2004), and estimation of nitrogen addition by bulk deposition into the 0-10 cm soil layer µg N g^{-1} sdw recalculated from bulk deposition (mg N l^{-1}; see Methods). Values are means ±SE (n = 3), b. d. l. indicates below detection limit.

	Soil N concentration		
	Before rain	After rain	Bulk deposition
$N\text{-}NH_4^+$	3.05±0.42	2.31±0.73	0.15±0.02
$N\text{-}NO_2^-$	0.35±0.03	1.74±0.86	0.007±0.002
$N\text{-}NO_3^-$	b. d. l.	0.40±0.26	0.18±0.04

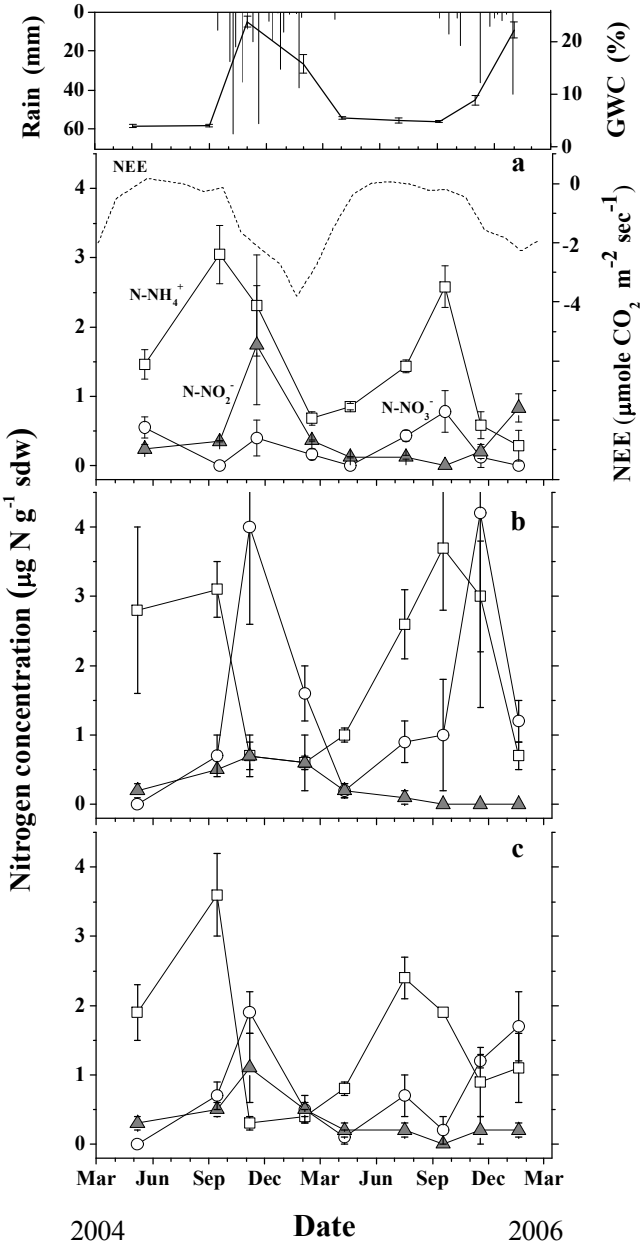


Figure 4.1 Dynamics (May 2004 - February 2006) of soil inorganic nitrogen, gravimetric soil water content (GWC) in mineral soil layer (0 - 10 cm) and Net Ecosystem CO_2 Exchange (NEE) in the Yatir afforestation (a) and surrounding shrubland, under the shrub canopy (b) and in the inter shrub bare soil (c). $N\text{-}NH_4^+$ - open cubes, $N\text{-}NO_3^-$ - open circles, $N\text{-}NO_2^-$ - filled triangles. Values represent the mean ±SE (n = 3, except for NEE, which is calculated from eddy covariance measurements above the canopy). Rain events distribution and quantity (mm) are shown by columns in the upper chart.

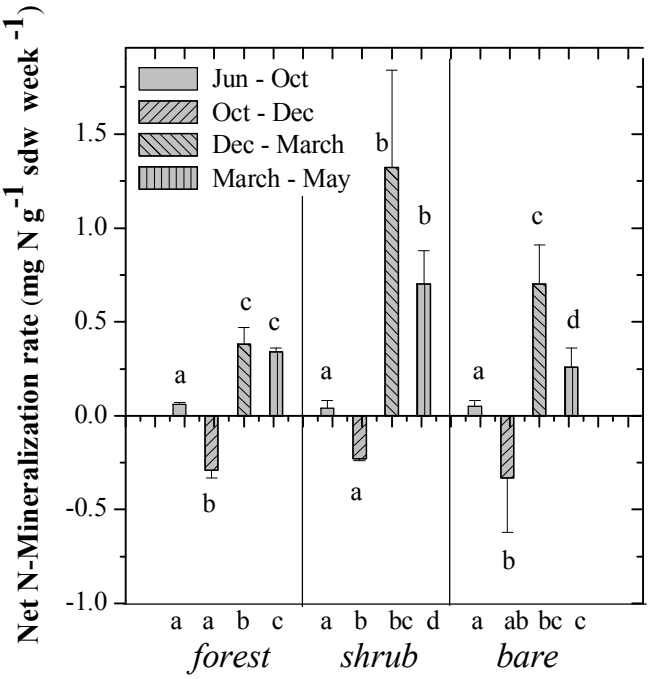


Figure 4.2 Net N-mineralization rates *in situ* (mean ±SE; $n = 3$) in the *forest* (F), *shrub* (SH), and *bare* (ISH) sub-plots in the dry (June - Oct), rewetting (Oct - Dec), and wet (Dec - March and March - May) seasons. Students' *t*-test significance ($p<0.05$) is indicated for the differences among seasons near the columns, and among sites for the same season at the bottom of the figure. Data are for the 2004-2005 season.

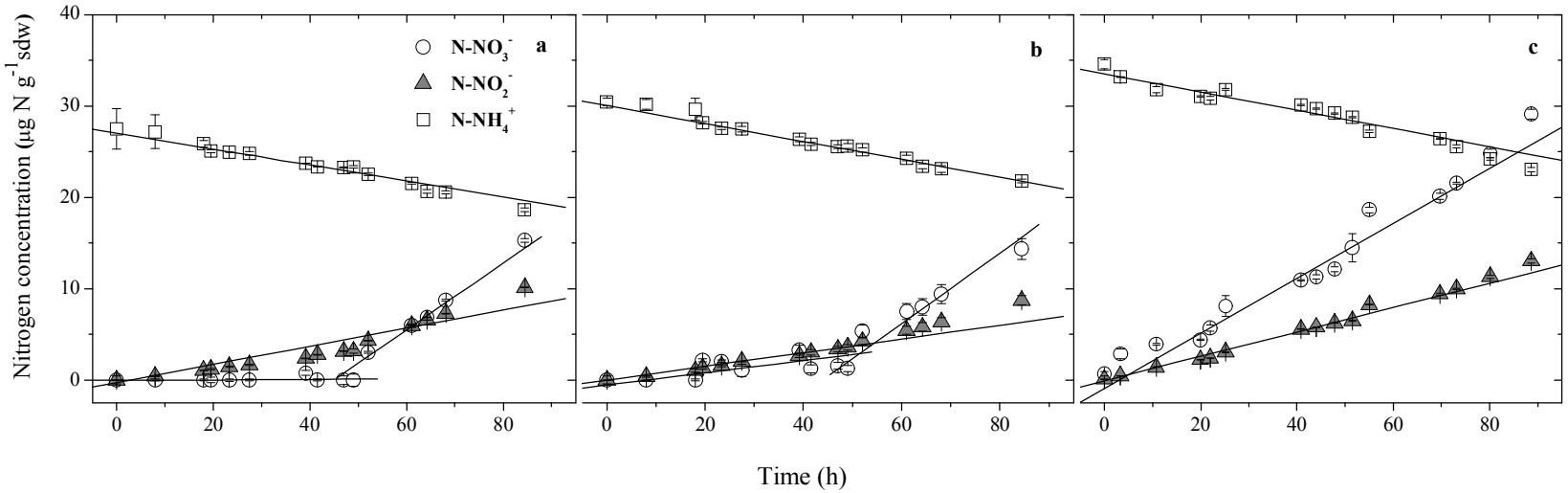


Figure 4.3 Nitrite and nitrate accumulation and ammonium consumption in soil slurry incubations from the Yatir afforestation during the dry period (**a**, October-05), rewetting period (**b**, December-05), wet period (**c**, February-06). Results represent the means ±SE (n = 3).

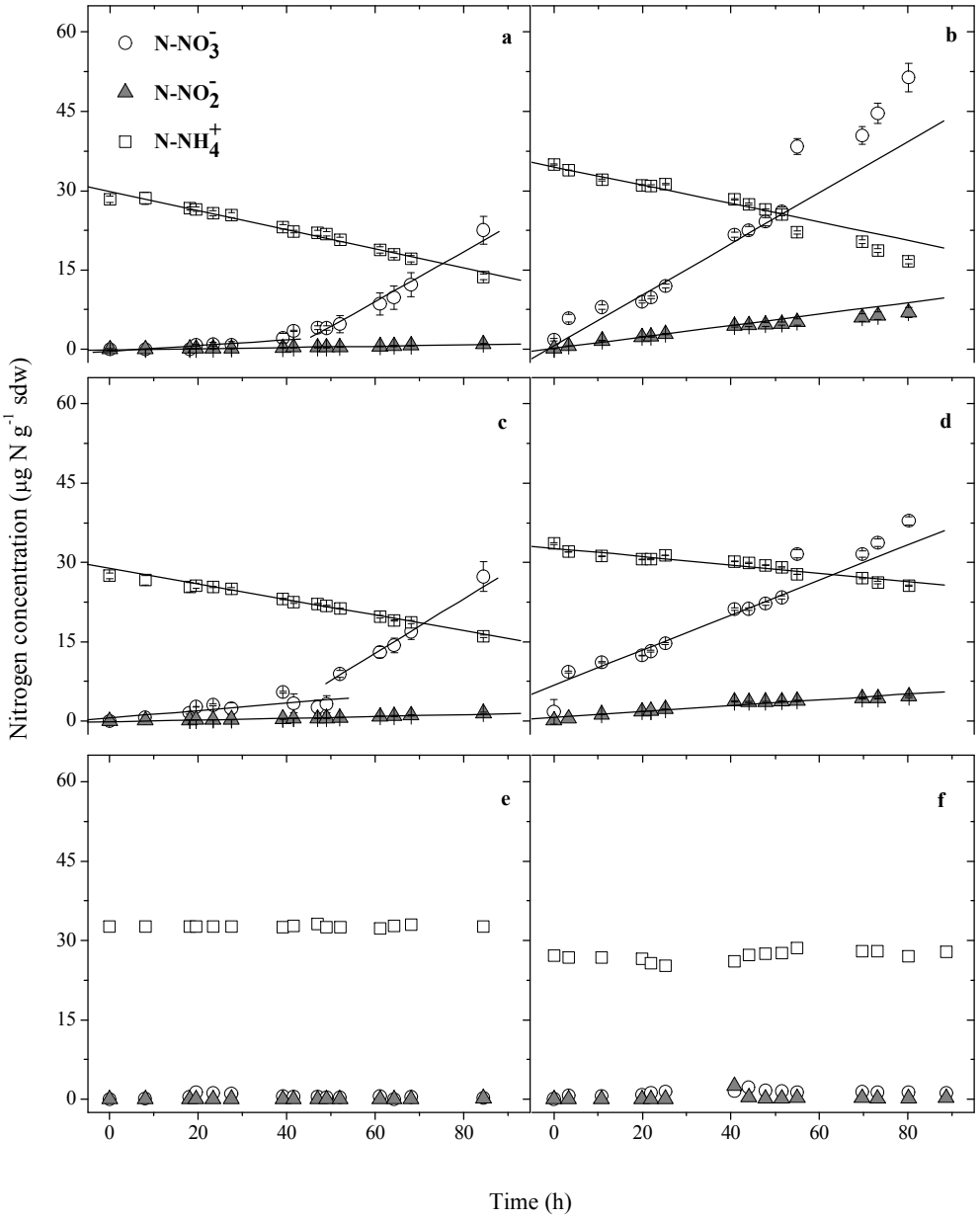


Figure 4.4 Nitrite and nitrate accumulation and ammonium consumption in soil slurry incubations from the shrubland during the dry period (a, c, from shrub and intershrub microsites, respectively, October-05), and during the wet period (b, d, from shrub and intershrub microsites, respectively, Feb-06), and in the control measurements (e, f, without and with autoclaved soils). Results represent means ±SE (n = 3)

Chapter 5

5 Effects of afforestation of semi-arid shrubland on biogenic NO emission from soil

5.1 Results

5.1.1 Laboratory results

The NO release rates from all soils had strong seasonal and spatial variability, as shown in Fig. 5.1 (a - c), where NO release rates are shown which have been sampled in October 2005, February 2006, and May 2006 from *forest*, *shrub*, and *bare* soils (all results obtained at T_{soil} = 28°C and $m_{NO,ref}$ = 0 ppb). Maximum NO release rates occurred in the soils sampled in October 2005 and the minimum NO release rates occurred in the soils sampled in February 2006. *Forest* and *shrub* soil samples from October 2005 had lower maximum release rates than the *bare* soils. The NO release rates were 63.7% and 58.5% lower for *forest* soils and *shrub* soils, respectively (1.32, 1.51 and 3.64 ng kg^{-1} s^{-1}, respectively for *forest*, *shrub,* and *bare* soils). All soils sampled in February 2006 had low maximum release rates of 0.12, 0.29, and 0.38 ng kg^{-1} s^{-1} for the *forest*, *shrub*, and *bare* soil, respectively. The February 2006 results indicated 68.4% and 23.7% lower maximum release rates for *forest* soils and soils, respectively, under *shrub,* compared with the *bare* soil, similar to the soils sampled in October 2005 (Fig. 5.1).

However, the soil samples from May 2006 showed a different NO release pattern. In all samples, the maximum release rates were higher than from the soils sampled in February 2006; however, the maximum release rates from the *shrub* soils were higher than those from *bare* and *forested* soils: 1.03, 0.74, and 0.35 ng kg^{-1} s^{-1}, respectively.

$WFPS_{opt}$ (i.e., WFPS at maximum NO release rates for a given soil) can be used as a first approximation for (a) the microbial process responsible for the emitted NO (e.g., anaerobic or aerobic metabolism) and (b) the maximum potential rate of the NO production process (Williams, 1992; Conrad, 2002). We found that soils sampled during different seasons (i.e., October 2005 - *dry-rewetting* season, February 2006 - *wet* season, and May 2006 - *drying* season) exhibited comparable $WFPS_{opt}$. The *forest*

samples in all incubations showed a $WFPS_{opt}$ at ~ 24%. The $WFPS_{opt}$ for NO release from the *shrub* soils changed from 26% (October 2005), to 32% (February 2006), and 23% (May 2006). Soil samples from the *bare* soils revealed a $WFPS_{opt}$ of 27% (October 2005 and February 2006), and 24% (May 2006). Remarkably, all soils incubated at 28°C showed a $WFPS_{opt}$ of between 23% and 30% irrespective of the vegetation or sampling seasons.

5.1.2 Temperature response of NO emission

The corresponding results for temperature response in different soils (obtained at $m_{NO,ref} = 0$ ppb) are shown in Fig. 5.2 (a – f). The reduction of incubation temperature (from 28°C to 18°C) of the Yatir forest soils sampled in October 2005 and May 2006, resulted in a similar shift of $WFPS_{opt}$, namely, from approximately 24% toward 40% (Fig. 5.2 a, b). In contrast, *bare* soils showed a decrease in the $WFPS_{opt}$ when incubated at 18°C, and corresponding $WFPS_{opt}$ values were shifted from 24 - 27% (28°C) toward 17 - 21% (18°C) (see Fig. 5.2 e, f). *Shrub* soils showed a shift of the $WFPS_{opt}$ from 26% (28°C) to 62% (18°C) for the October 2005 samples and from 23% (28°C) to 32% (18°C) for the May 2006 samples (Fig. 5.2 c, d).

For the sake of simplicity, we would like to consider the observed temperature dependence of J_{NO} for the different soil samples in terms of $Q_{10}^* = Q_{10}(WFPS_{opt})$, calculated as the ratio of $J_{NO}(WFPS_{opt}, 28°C)$ and $J_{NO}(WFPS_{opt}, 18°C)$. The Q_{10}^* value was strongly dependent on the soil origin (i.e., *forest*, *shrub,* and *bare* soils) and the sampling period. Whereas the *forest* soils from October 2005 showed a Q_{10}^* of 4.1, the *forest* soil samples from May 2006 showed virtually no temperature response ($Q_{10}^* = 1.1$) (Fig. 5.2 a, b). The *shrub* soils revealed a Q_{10}^* from 4.4 to 3.0, and the *bare* soils revealed a Q_{10}^* from 3.5 to 0.95 for the October 2005 and May 2006 samples, respectively (Fig. 5.2 c, d, e, and f).

Integration of the overall mass of NO released from the soils during the entire incubation with different temperatures revealed the following results: *forest* soils showed a NO release reduction by a factor of 3.9 and 0.9, *shrub* soils showed a reduction of 10.5 and 2.9, and *bare* soil showed a reduction of 3.1 and 0.8 for the October 2005 and May 2006 soil samples, respectively.

To summarize, a reduction of the incubation temperature by 10°C resulted in changes in both the $WFPS_{opt}$ and the Q_{10}^{*} of the different soils on both spatial (i.e., between soils with different vegetation cover) and temporal (i.e., seasonal) scales (Fig. 5.2).

5.1.3 Upscaling to ecosystem scale

In order to evaluate the effect of afforestation of the shrubland on biogenic soil NO emissions, we up-scaled the laboratory measurements, using field measured soil temperature and water content. Results of our up-scaling exercise, namely, annual cycles of the daily F_{NO}-estimates for *forest*, *shrub*, and *bare* soils are shown in Fig. 5.3 (a to c), together with the annual course of mean daily soil temperatures and soil moistures (WFPS). The results from Fig. 5.3 were integrated for each of the three 'seasons' and given as mean daily sums (to fit with the time scale of the environmental measurements) in Table 5.1.

Up-scaled NO emissions were found to be sensitive to measured T_{soil}, WFPS, specific soils, and exhibited distinctive seasonal patterns. Maximum NO emissions were calculated during the *dry-rewetting* season, when the WFPS was stable (around 14%), and T_{soil} increased above 20°C. The highest emission rates were calculated from the *bare* soils and the minimum emission rates were from *forest* soils (Fig. 5.3; Table 5.1). We were able to show the emission peak after the first significant rain event (November 21, 2005) and subsequent drying in all three studied ecosystems (Fig. 5.3, "December"). An additional peak of NO emission occurred during the *drying* period after the *wet* season, along with the rise in soil temperature (Fig. 5.3b, c, "May – June"). However, this peak was almost absent in the *forest* soil. Low emissions during the *wet* season were calculated for all ecosystems, because of (a) low release rates observed during the corresponding laboratory incubations (see Fig. 5.1), and (b) high WFPS and low soil temperatures measured in the field (Fig. 5.3).

Seasonal trends in mean daily NO emissions and mean annual NO emissions ($A(NO)_i$) of each soil cover type (i = *forest*, *shrub*, *bare*; Table 5.1) were calculated by summing up individual daily $D(NO)_i = F(NO)_i \times (8.64 \times 10^{-2})$ emission rates over the corresponding number of days (j) of each season, considering the relative free soil area

s_i (i.e., after subtracting the estimated area of rocks, stamps, and stems, see section 2.8) of the individual microsites:

$$A(NO)_i = s_i \times \left(\sum_{j=1}^{j=158} D(NO)_{dry-rewetting,i,j} + \sum_{j=1}^{j=114} D(NO)_{wet,i,j} + \sum_{j=1}^{j=93} D(NO)_{drying,i,j} \right) \times 10^{-2} \quad (5.1)$$

where i = *forest*, *shrub*, *bare*, s_i is in %, $D(NO)_i$ is in mg m^{-2} d^{-1}, and $F(NO)_i$ is in ng m^{-2} s^{-1} (in terms of the mass of nitrogen). The season- and area-weighted mean annual biogenic NO emission for the *forest* soil was 0.11 kg ha^{-1} y^{-1}, whereas emission from surrounding shrublands (*shrub* covered and *bare* soils) was 0.32 kg ha^{-1} y^{-1} (Table 5.1).

Annual NO emission rates, calculated using microsite specific values based on limited estimates of k using our soil samples showed different results. The season- and area-weighted mean annual biogenic NO emission for the *forest* soil was 0.20 kg h^{-1} y^{-1} whereas emission form surrounding shrubland was 0.24 kg h^{-1} y^{-1} (Table 5.1)

To eliminate k related differences between the estimation of annual NO emission rates we calculated "gross" emission rates using our lab measured J_{NO}. The season- and area-weighted mean annual biogenic NO emission for the *forest* soils was 11.44 kg h^{-1} y^{-1} whereas emission from the shrubland was 28.15 kg h^{-1} y^{-1} (Table 5.1).

5.2 Discussion

The soils from the Yatir forest and surrounding "background" shrubland were analyzed to examine the effects of afforestation on potential NO emission. All soils types examined showed seasonal and spatial variability of NO emission patterns and rates. This seasonal pattern was used, together with field soil moisture and temperature measurements to upscale NO emission in ecosystem scale. We found that afforestation in the semi-arid northern Negev shrubland reduces soil NO emissions by more than 35% in comparison to the native shrubland.

5.2.1 Effects of environmental variables on NO emission

The laboratory incubations of the soils revealed a seasonal pattern, with the highest NO release rates from soils sampled in the *dry-rewetting* season, and the lowest release rates from soil samples in the middle of the *wet* season (Fig. 5.1). These

seasonal trends can be explained by corresponding patterns of soil temperature and soil water content and assuming continuous adaptation of the soil microbial community to the changing conditions (Johansson and Sanhueza, 1988; Harris et al., 1996; Scholes et al., 1997; Smith et al., 2003). The estimate from our up-scaling approach of high ecosystem-scale NO emissions after rewetting of dry soils is consistent with other studies of semi-arid ecosystems, which have also attributed high NO emission to the increase in water availability for microbial activity (Davidson et al., 1993; Meixner et al., 1997; Hartley and Schlesinger, 2000; Martin et al., 2003). We attribute both the low NO release rates derived from laboratory incubations and the low NO emission rates obtained from up-scaling during the *wet* season to the fact that during this season (a) the soils are too wet to allow good aeration, and (b) the soil temperature is markedly below the optimum for microbial activity (WFPS>40%; T<15°C; Meixner and Yang, (2006); Conrad, (1996); Williams, (1992)). Decreases of NO flux during high soil moisture regimes were also shown by Rosenkranz et al. (2006) and were partly attributed to enhanced aerobic NO consumption by heterotrophic nitrifiers. Alternatively, the decrease of NO flux with increasing soil water content could also be explained by a decrease in nitrification activity due to reduced diffusion of the oxygen into the soil. However, this is inconsistent with in situ nitrification activity and mineralization rates in soils that showed opposite trends, namely, an increase to ~0.4 μg g^{-1} $week^{-1}$ (in terms of soil dry weight) from virtually zero, with increasing soil water content (Chapter 4). During the *drying* season, soils showed intermediate NO emission rates; lower than during *dry-rewetting* seasons and higher than *wet* season rates (Table 5.1). We can explain these findings by the increase in soil temperature, together with a decrease in the soil water content towards optimum conditions ($WFPS_{opt}$) for NO emission. We hypothesize that the seasonal pattern of NO emission from soils is due to gradual changes in both soil temperature and soil water content, which induce activation of different microbial sub-populations (Avrahami and Bohannan, 2007). During the start of the *rewetting* season, nitrifying bacteria have a temporal advantage regarding N supply because of their fast recovery from drought and because of their relative tolerance to high-temperatures (Hastings et al., 2000; Chapter 4).

Two mechanisms for NO formation in soils from seasonally dry tropical forests have been proposed by Davidson et al. (1993), namely, oxidation of ammonia and the

chemo-denitrification of HNO_2 (nitrous acid), an intermediate of both the nitrification and denitrification processes. Both mechanisms would take place in our ecosystem. Our previous study of soils representing the *dry-rewetting* season from our ecosystems indicated high activity of ammonia oxidizing microorganisms and a temporal delay between the ammonia and nitrite oxidation (Chapter 4). The second proposed mechanism for NO formation is self-decomposition of HNO_2 and the possible reaction of HNO_2 with soil organic matter. This NO formation pathway is generally expected to be more important in acidic soils (pH < 5.5; van Dijk et al., (2002)). However, during nitrification, both nitrite (NO_2^-) and H^+ may accumulate and possibly result in acidified microsites, even in alkaline soils, where conditions can favor the formation of HNO_2 and therefore NO. Finally, nitrite was shown to enhance NO production in the soils (Davidson et al., 1993). From the above, we can conclude that enhanced NO emission during the *dry-rewetting* season is consistent with both the presence of HNO_2 and the relatively high ammonia concentration in the soil.

The possibility of NO uptake by the soil was noted in the past and was assumed to be an integral part of the N metabolism in soil; a corresponding concept of a NO compensation mixing ratio in soils was defined by Conrad (1994). According to eq. (2.8), the NO compensation mixing ratio is given by $m_{NO,comp} = P/k$ (c.f. Remde et al. (1993)). From our deduced k-values (see section 2.8) and from the range of J_{NO} (observed at $m_{NO,ref} = 0$ ppb; Fig. 5.1), we estimated the NO compensation mixing ratios for *forest*, *shrub*, and *bare* soils to be 149.3, 170.8, and 413.6 ppb NO. These NO compensation mixing ratios fall within the wide range of values reported from laboratory study (15 to 600 ppb; Remde et al., 1989; 1993). As discussed previously (Otter et al. (1999) and references within) this values are much higher than those found in field studies. A sensitivity test using our data indicated that up scaling of NO incubation data to the field scale, critically depended on considering the large seasonal changes in J_{NO} (Fig. 5.3) and reliable estimate of k, and is less sensitive to variations in WFPS and temperature. In our scaling up experiment, there was no indication of no nitric oxide uptake fluxes (NO deposition), which is most likely because the low ambient NO mixing ratios (annual mean 2.39±0.56 ppb, see section 2.8) never exceeded the estimated NO compensation mixing ratio.

Similar values of $WFPS_{opt}$ for *forest*, *shrub*, and *bare* soils (Fig. 5.1) pointing towards similar composition of microbial populations in these soils. Thus, the difference in NO release rates among sites and seasons might be explained by changes in the microbial activity rates and the influence of plant activity, such as N uptake from the soil and microsite conditions, such as carbon availability for heterotrophic microbes (Meixner, et al., 1997; Hartley and Schlesinger, 2000; Hackl et al., 2004; Cookson et al., 2007).

Rates of NO release in soils with different vegetation cover revealed different dependencies on soil temperature (Fig. 5.2). The observed Q_{10}* of the NO release appears to be not only soil specific, but also season specific. Similar seasonal and spatial variations in Q_{10} were recently shown for CO_2 efflux in a Sierra Nevada forest plantation (Xu and Qi, 2001). Soil temperature controls on the NO emission (e.g., Q_{10}) seem to be more important for short-term variations of the NO release, whereas the magnitude of the biogenic release rates is predominantly controlled by other seasonal factors such as soil water content and N availability (Meixner and Yang, 2006). From Figure 5.2, it can be seen that for our *forest*, *shrub*, and *bare* soils, there is no simple relationship between soil temperature, and NO release rates cannot be simply explained by $Q_{10} = 2$ or any alternative constant (Godde and Conrad, 1999; Brierley et al., 2001; Fierer et al., 2003; Wang et al., 2004). The Q_{10}* calculated for our soils ranged from 0.95 up to 4.4.

Our results show that when considering the annual time-scale, inter-seasonal variations in soil NO release, as well as factors influencing it, must be considered. When taken into account, the average soil NO release rates for our ecosystems were on the lower end of published NO release rate estimates in other semi-arid biomes (see reviews by Davidson and Kingerlee, 1997 and Meixner and Yang, 2006). Specifically, published NO release estimates for semi-arid and arid ecosystems range from 1.8 to 3.8 ng m^{-2} s^{-1} for dry tropical forests (Davidson et al., 1993), 0.1 to 3.7 ng m^{-2} s^{-1} for Miombo woodland and grassland in Zimbabwe (Kirkman et al., 2001), 0.3 to 21.9 ng m^{-2} s^{-1} for South African savannas (Parsons et al., 1996), and 0 to 4.9 ng m^{-2} s^{-1} for the Chihuahuan Desert, New Mexico (Hartley and Schlesinger, 2000). The annual, seasonally weighted mean NO release values in our ecosystems were 0.3, 0.4 and 1.6 ng m^{-2} s^{-1} for the *forest*, *shrub* and *bare* soils, respectively (converted from Table 5.1).

5.2.2 Influence of afforestation on the NO emission

Information on the influence of land use change on NO emission, including type of vegetation covers or proportion among microsites (e.g., *shrub* and *bare* types) is limited at present. NO emissions were shown to decrease with decreasing soil cover (Davidson et al., 1993; Martin and Asner, 2005), grassland and savanna soils were shown to be stronger sources of NO emission than soils with greater vegetation cover (Williams, 1992; Kirkman et al., 2001). Note that in the shrubland, the emission estimate is sensitive to the proportions of *shrub* and *bare* soil covers since both had different average release rates (0.04 and 0.14 mg m^{-2} d^{-1}, respectively). The main effect observed here is therefore in going from bare soil to the forest soil (release rate of 0.03 mg m^{-2} d^{-1}). Considering the proportion of land cover by shrubs and bare soil (see Table 5.1), the up-scaled rates for our particular case indicated ~65% apparent reduction in NO release rates (from 0.32 to 0.11 kg ha^{-1} y^{-1}) associated with the afforestation.

However, when using lab estimated *k* values and gross emission values, large differences in NO emission reduction were observed. Use of lab estimated *k* values for upscaling procedure revealed only 17% reduction (0.24 to 0.20 kg ha^{-1} y^{-1}) of annual NO emission and "gross" emission rates showed 59% (28.15 to 11.44 kg ha^{-1} y^{-1}) of reduction. These differences in estimation of afforestation effect reveal need in further research and better understanding of the processes that lead to NO consumption by soils and particularly better estimation of the NO consumption factor (*k*).

However, we can point on the strong tendency toward afforestation driven reduction of soil NO emissions. We speculate that the reduction in NO flux is due to increased N uptake by the forest trees (there was ~2.5-fold increase in ecosystem organic carbon stock, associated with afforestation), reducing soil N availability for nitrification (Jackson et al., 1989; Zak et al., 1990; Kaye and Hart, 1997; Grünzweig et al., 2007).

The importance of the seasonal effect was especially apparent in up-scaling the laboratory results to the ecosystem scale (Fig. 5.3). For example, if we would calculate the annual NO release rates for the Yatir forest with the data from the laboratory incubations of the October 2005 samples alone, we would overestimate the annual release rates by 48%. Similarly, if we would use data from the February 2006 soil samples alone, we would underestimate the annual release rates by 73%.

Because of the observed sensitivity to sampling location, date, soil cover and consumption rate in the semi-arid ecosystems, it seems important that further studies using soil sample incubations should consider (a) at least one soil sample per distinct season, (b) incubation of the samples under corresponding field conditions (e.g., average field water content and soil temperature at the time of soil sampling) and (c) explicit determination of the *k*-values.

Table 5.1 Mean daily ($D(NO)_i$) and annual ($A(NO)_i$) NO emission rates for different seasons and soils of the Yatir forest and shrubland ecosystems. Number of days of each season (j in eq.5.1 used for upscaling), where *dry-rewetting=20 July 2005 to 24 December 2005*; *wet=25 December 2005 to 17 April 2006; drying=18 April 2006 to 19 July 2006*. The type of micro-site (i used in eq. 5.1 for upscaling): *forest, shrub* (i.e. under-shrubs samples*), bare* (inter-shrub samples), are indicated and the under-shrub and inter-shrub components are integrated into the *shrubland* ecosystem; s_i is the respective free soil surface area (corrected for contribution of rocks, stamps, and stems). Estimates are based on lab incubations of soil samples collected in the different seasons and microsites using a mean literature *k* value for semi-arid environment, or (given in parentheses) using microsite-specific values based on limited estimates of *k* using our soil samples [a] and using gross emission rates [b] (see section 2.8).

		Seasonal mean of daily NO emissions $D(NO)_I$ [mg m^{-2} d^{-1}] microsite (*i*)			
Season	Days (*j*)	*shrub*	*bare*	*shrubland*	*forest*
Area contribution s_i (in %) **:**		25±3	56±2	81±3	86±5
dry-rewetting	158	0.06 (0.14) [a] (1.66) [b]	0.25 (0.17) [a] (11.73) [b]	0.16 (0.13) [a] (13.40) [b]	0.06 (0.12) [a] (5.09) [b]
Wet	114	0.01 (0.00) [a] (0.15) [b]	0.00 (0.00) [a] (0.66) [b]	0.00 (0.00) [a] (0.81) [b]	0.00 (0.00) [a] (0.54) [b]
Drying	93	0.05 (0.08) [a] (1.39) [b]	0.11 (0.02) [a] (5.20) [b]	0.07 (0.07) [a] (6.59) [b]	0.03 (0.04) [a] (3.01) [b]
Annual rate	365	0.04 (0.08) [a] (1.12) [b]	0.14 (0.08) [a] (6.61) [b]	0.09 (0.06) [a] (7.73) [b]	0.03 (0.06) [a] (3.14) [b]
Annual mean NO emission $A(NO)_i$ (kg $ha^{-1}y^{-1}$) (season & area weighted)				**0.32** **(0.24)** [a] **(28.15)** [b]	**0.11** **(0.20)** [a] **(11.44)** [b]

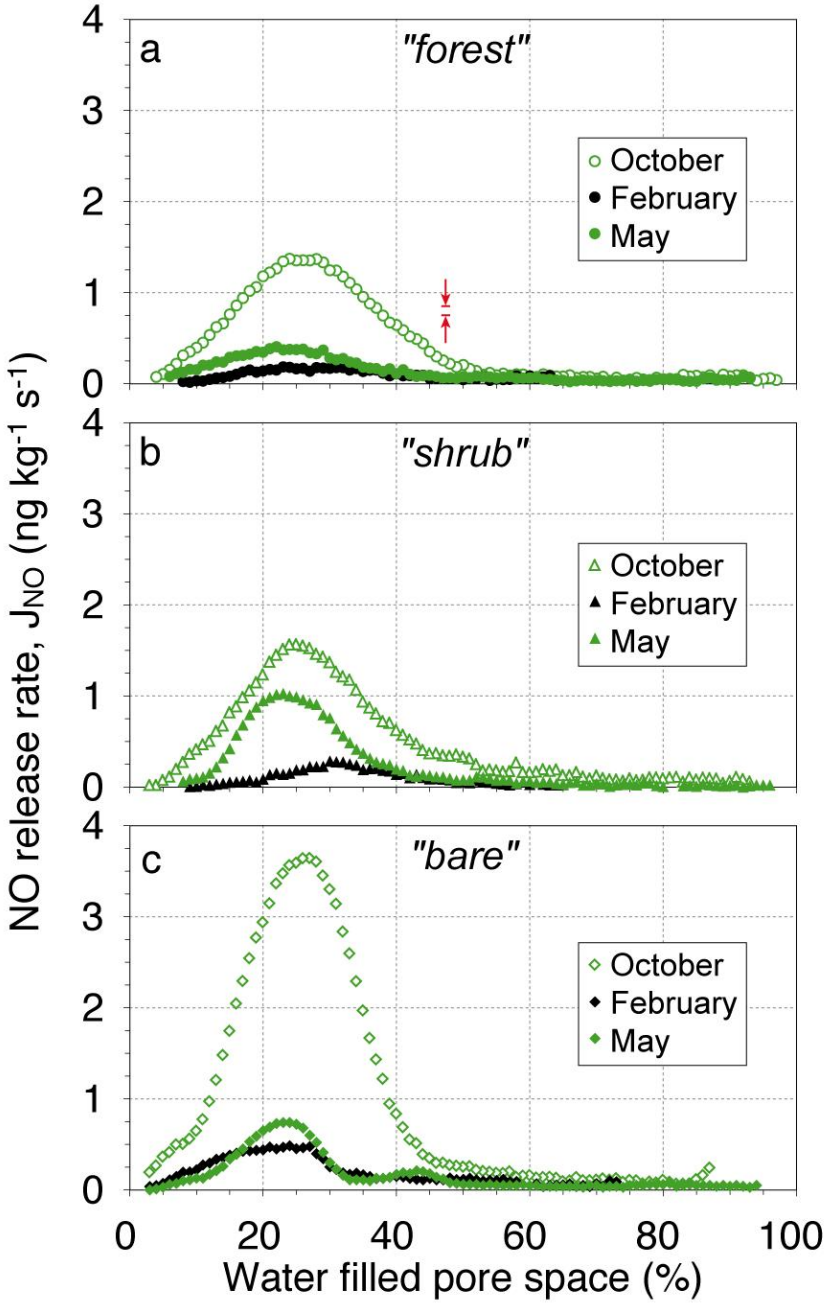


Figure 5.1 Seasonal variability of the release rate J_{NO} (at T_{soil} = 28°C and $m_{NO,ref}$ = 0 ppb) from soil samples of (a) Yatir forest soil (*forest*), (b) surrounding shrubland soil (under shrub canopy; *shrub*), and (c) inter-shrub *bare* soil; open circles, filled squares, and open triangles represent soils sampled in October 2005, February 2006, and May 2006, respectively. A representative error of J_{NO} measurements (0.05 ng kg^{-1} s^{-1}, in terms of mass of N; see section 2.9) is shown in Fig. 5.1a.

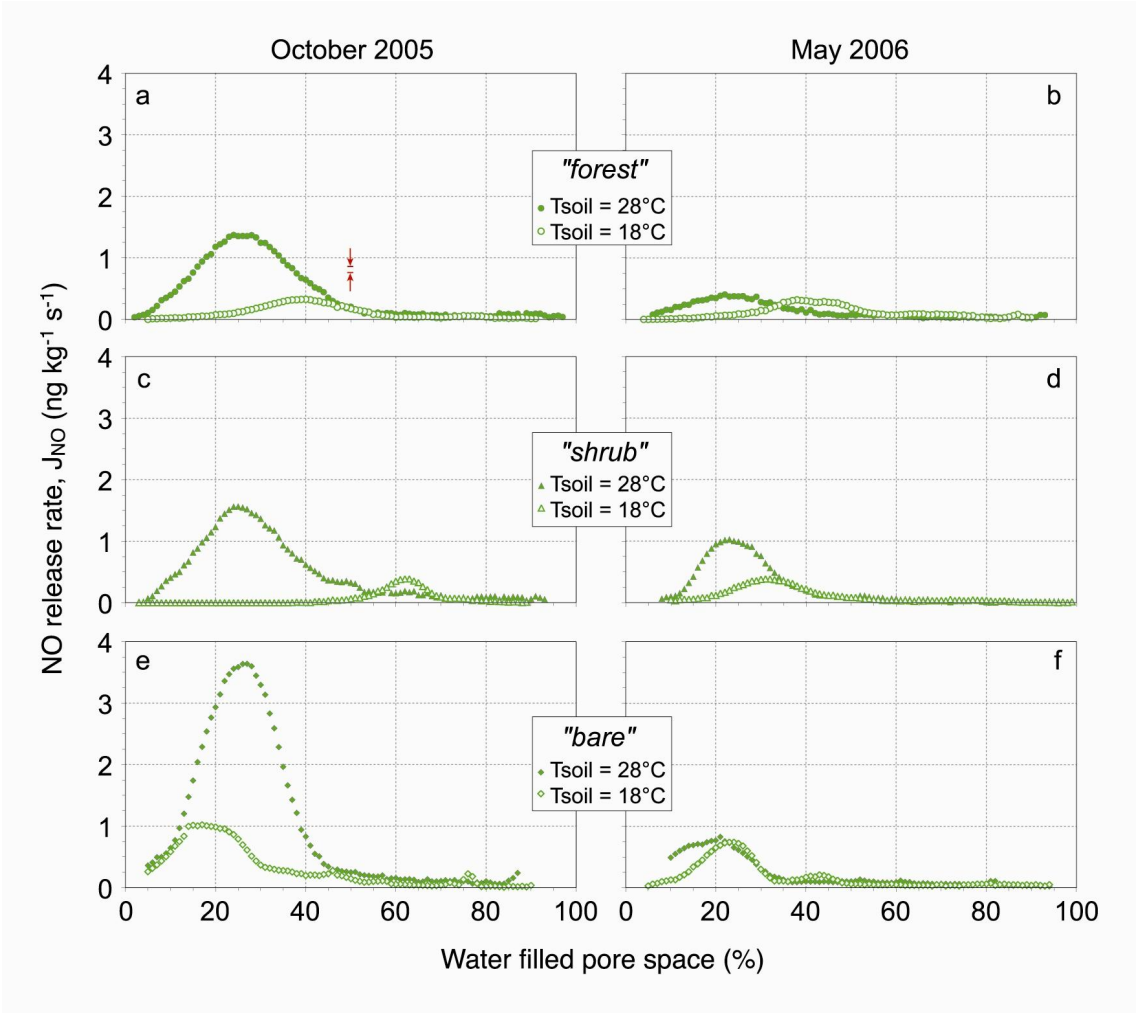


Figure 5.2 Temperature dependence of NO release rates J_{NO} (ng kg^{-1} s^{-1}; in terms of mass of N) of soils sampled in October 2005 and May 2006 and incubated at $m_{NO,ref}$ = 0 ppb: Yatir forest (*forest*; a, b); under shrub canopy (*shrub*; c, d), and inter-shrub bare soils (*bare*; e, f). Open circles represent incubations at T_{soil} = 28°C and filled circles at T_{soil} = 18°C, respectively. A representative error of J_{NO} measurements (0.05 ng kg^{-1} s^{-1}, see section 2.9) is shown in Fig. 5.2a.

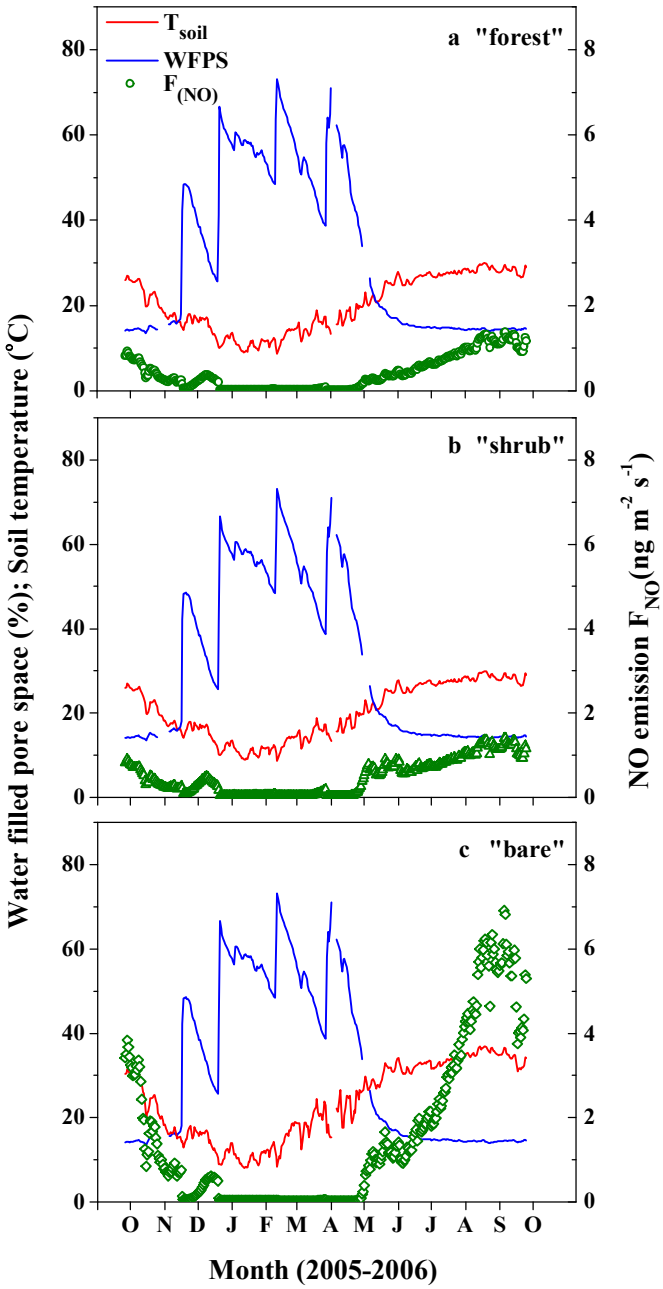


Figure 5.3 Results of up-scaling NO emission fluxes from laboratory incubations of soil samples of the Yatir forest ecosystem based on measurements of soil water content (at 5 cm depth) and soil temperature (at 6 cm depth) in the field; (a) Yatir forest (*forest*), (b) under shrub canopy (*shrub*), and (c) inter-shrub (*bare*) soil. Open squares indicate soil temperature (°C), dots indicate the NO emission flux (ng m^{-2} s^{-1}, in terms of mass of N), and the line represents WFPS (%).

Chapter 6

6 General discussion

6.1 Changes in the N balance associated with afforestation

For studying afforestation-induced changes in the ecosystem N cycle we investigated and compared the N badget of the Yatir forest with that of the surrounding shrubland. Additionally, we investigated the main patterns of, and controls on, the N cycle, including dynamics of litter decomposition, nitrification, and nitrogen oxide emissions. Our initial working hypotheses assumed that there must be an increase in the N input to the ecosystem and acceleration of the nitrogen cycle in the soil. Alternatively, we hypothesized that if this is not the case, increasing N demand following increasing in carbon storage (Grünzweig et al., 2007) must be reflected in the increase in NUE and N recycling within the plant tissues. We used a ecological/biogeochemical approach at the ecosystem-scale, and attempted concentrate on important gaps in our understanding of ecosystem functioning.

We showed that despite the large biomass buildup in the afforested ecosystem, the main components of the N budget, as well as the net N balance, did not change considerably (Chapter 3). The forest exhibited a high NUE, slow N turnover and overall a slower and tighter N cycle (Table 3.1; Table 3.2). The slowing down of the N cycle rates lead us to characterize the Yatir forest as an ecosystem with “closer” and more conservative N relations. The main differences with other studied ecosystem were: decrease in litter decomposition and net mineralization rates; small increase of the N input but focused toward the wet, active season; and a small decrease in N output. The latter changes, however, neglected a decrease in the biological N fixation and biocrust cover (Chapter 3; Fig. 3.1). Despite the fact that the main components of the N cycle did not change much, the forest has a relatively high primary productivity (Grünzweig et al., 2003). From the discussion above (see section 3.2) we can conclude that the Yatir forest productivity seems to be limited more by water than by N. This conclusion is partly supported by essentially ‘neutral’ N balance of the ecosystems (Chapter 3). The relatively larger water limitation on the ecosystems originates, at least partly, in a pulsed-pattern of water availability (Maseyk, 2006; Chapter 4).

Inorganic N accumulates in the soils over the dry summer (Chapter 4) and with the onset of the active wet season it is rapidly used by the plants. A decrease in the inorganic N concentration in the soils during the wet season, together with fast mineralization rates, point to a fast flow of N through the ecosystem (Chapter 4). The shrubland ecosystem, in contrast to the forest, does not use all available N and, although it has enough resources (in terms of N), it has lower productivity. The lower NUE of the shrubland may result in the loss of N to the lower soil horizons where it becomes unavailable for plants, a process that was shown to take place in semi-arid western USA (Walvoord et al., 2003). In order to investigate this possibility in our ecosystem further studies are needed.

While we observed successful operation of the pine afforestation in Yatir, there is lack of natural growth of pine trees in the northern Negev and the Yatir forest is show very slow expansion. This can be explained mainly by poor seedling establishment in this region (c.f. Bonneh, 2000). Indeed, near our research site in Yatir forest we observed large numbers of pine seedling development, but these seedlings did not appear to survive the combination of the long dry summer season and livestock grazing. In this research we did not address the grazing aspects and it's consequences for ecosystems functioning. Note however that grazing of the forested and un-forested sites was essentially of similar magnitude in our area.

6.2 Controls on the N cycle

The activity patterns of the studied ecosystems (both soil and plant) followed patterns of water availability. The annual cycle in our ecosystems can be divided into two main seasons. First is a shorter wet season with relatively low average temperatures and relatively high soil water content. Second is long dry season, during which the average temperatures are high and water content of the soil is very low (see Chapter 5 for annual patterns of soil water content and temperature, Fig. 5.3). These seasons have distinct patterns of biological activity. During summer drought the soil and plant processes are extremely slow due to strong water limitation (see Chapter 4, Fig 4.1). During the wet season water availability is increased, but temperature decreases to relatively low values of ~10°C, which possibly limit the microbial and at less extent, plant activity. This interplay between the limiting factors of low water or low

temperature plays a major role in regulation of the nitrogen cycle in this semi-arid environment. During the dry season nitrogen accumulates in the soil profile mainly due to ongoing dry deposition and mineralization processes (although the latter have very low rates) while the nitrogen uptake by biota is restricted due to extreme drought and high temperature conditions (~5% v/v water content and average of ~35°C in 0 - 10 cm soil layer). Accumulation of the inorganic N in the soil profile induces two main processes. Firstly, the high inorganic nitrogen (mainly in the ammonium form, ~5.0±0.1 kg N ha^{-1} in forest and 2.9±1.2 kg N ha^{-1} in shrubland, Chapter 3; Table 3.1) provides preferable conditions for the AOB populations (Chapter 4). This was reflected in our observations of nitrite accumulation in the 0 - 10 cm soil layer (Chapter 4) and increasing NO emissions (Chapter 5). The latter phenomena possibly occur in response to a positive feedback between ammonium oxidation and NO production by AOB, which was discussed in more detail in Chapter 5. Secondly, the presence of highly available nitrogen in the soil at the start of the wet season must have a positive influence on plant activity. The Aleppo pine trees are growing their foliage during summer (Bonneh, 2000), and an additional nitrogen source at the start of the active season likely helps maintain high forest productivity. Moreover, nitrite accumulation in the soils can have an inhibiting effect on the soil microbial communities, decreasing the competition with the pine trees for the available resource, and possibly leads to synchronization between the nitrogen demand and availability in the plants.

Chapter 7

7 Conclusions

The afforestation of dry lands can be used as a tool to both combat desertification and sequester carbon from the atmosphere (Pacala and Sokolow, 2004). In this research we made some of the first investigations into the afforestation driven changes in the ecosystem N cycle in this environment. In this framework, we compared the N budgets of the Yatir forest and surrounding shrubland ecosystems. The forest was found to have slower N cycling and an increase in the N input during the season of activity. The forest ecosystem also showed a sharp increase in NUE, N MRT and N productivity, both in aboveground and belowground components of the ecosystem. Our results demonstrate how small "adjustments" in the ecosystem N cycle can help support the almost 2.5 fold increase in the carbon stock of the ecosystem, without any significant additional nitrogen.

We investigated controls on the nitrification process and gaseous nitrogen oxide emissions from the ecosystems. We observed unexpected fast nitrite accumulation in the soil of semi-arid pine afforestation at the end of the seasonal drought period. Laboratory incubations indicated that the nitrite accumulation reflected a temporal delay between the oxidation of ammonium and nitrite, probably due to differences in drought tolerance and recovery rates of AOB versus NOB microbial populations. These effects resulted in a delay in the onset of nitrate accumulation at the beginning of the wet season in the soils of this semi-arid afforestation. We hypothesized that such delay may improve the synchronization of N availability with peak plant activity, and contribute to the high ecosystem NUE and N productivity observed in this system. We also note that the presence of nitrite in the soil may have inhibitory effects on the microbial immobilization of inorganic N due to nitrite toxicity even at low concentrations, an effect that requires further study.

Soils from the Yatir forest and surrounding shrubland were analyzed to examine the effects of afforestation on NO emission. All studied soils showed considerable variability in the seasonal and spatial patterns of NO release rates and NO emissions. The seasonal pattern of laboratory-derived NO release rates were used, together with data from soil moisture and soil temperature measurements in the field, to up-scale NO

emissions to the ecosystem scale. We found that afforestation in the semi-arid northern Negev shrubland reduced soil NO emissions between 16.7 and 65.6% in comparison with the native shrubland (depend on estimation approach). The average annual NO emissions rates from *forest*, *shrub*, and *bare* soils were at the lower range of previous estimates for biogenic NO emission from arid and semi-arid ecosystems. We emphasize the predictive power of our up-scaling procedure, which is based on (a) seasonal soil sampling, (b) incubation under a corresponding soil moisture, temperature, and ambient NO mixing ratio, and (c) application of a comprehensive and validated up-scaling algorithm.

As the Mediterranean region is expected to experience decreasing precipitation trends with future global warming (IPCC, 2007), the effects observed in this semi-arid forest may well be applicable to increasingly larger areas in the future. Furthermore, the results obtained in this study show significant adjustments in the dry-land pine forest that help maintain relatively high productivity. This differs from predictions based on episodic drought years in wetter climates, often used as a basis for future scenarios.

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