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The effect of phosphorus amendments on nitrogen fixation and growth of trees on salt-affected croplands in the lower reaches of Amu Darya, Uzbekistan

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von
DILFUZA DJUMAEVA
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To my husband John Lamers,

our daughter Nicole Anna Cecilia,

and our son Daniel Hendricus Ronald

ABSTRACT

Afforestation, particularly with the use of N_2-fixing trees (NFTs), is an option for ecological restoration of salinized, irrigated croplands in the lower reaches of the Amu Darya River. But current knowledge of enhanced juvenile tree growth and their N_2 fixation rates is sparse for the marginal irrigated croplands of the Khorezm Region of Uzbekistan. A superior understanding would increase productivity of such lands, improve soil fertility status and increase profits for farmers. A two-factorial field experiment was therefore conducted during 2006-2008 to compare the effect of three phosphorus (P) amendments on N_2 fixation, biomass and growth rates of actinorhizal *Elaeagnus angustifolia* L. and leguminous *Robinia pseudoacacia* L. N_2 fixation was quantified through ^{15}N natural abundance, based on foliar and whole-tree sampling against non-N-fixing *Gleditsia triacanthos* L. The P rates included: (i) high-P (90 kg P ha^{-1}), (ii) low-P (45 kg P ha^{-1}), and (iii) no P applied (0-P). With high-P, N_2 fixation by *E. angustifolia* increased by 81% and almost doubled for *R. pseudoacacia* when compared to 0-P. At a tree density of 5,714 trees ha^{-1}, N_2 fixed with high-P increased from an initial value of 64 kg ha^{-1} to 807 kg ha^{-1} after three years in *E. angustifolia*, and from 9 kg ha^{-1} to 155 kg ha^{-1} in *R. pseudoacacia* stands. The P-effect was inconsistent when analyzing absolute growth and biomass increase. Compared to 0-P, high-P increased total biomass, total above ground biomass and biomass of different tree fractions, but the increments in absolute growth were statistically insignificant. In contrast, high-P significantly increased relative growth rates of height for *E. angustifolia*, and the unit production rate and nitrogen productivity for *R. pseudoacacia*. Hence tree growth analyses should combine absolute and relative growth to gain a full insight.

N_2 fixation of *E. angustifolia*, the species with the highest potential in the field trial, was also quantified in lysimeters to eliminate inaccuracies that can occur when harvesting large trees in open fields. Here we used more than one reference species and two assessment methods, the ^{15}N-enrichment technique (^{15}NET) and the A-value (AV). The non-N-fixers *G. triacanthos* and *Ulmus pumila* L. served as a reference. Twenty kg N ha^{-1} of 5 atom % ^{15}N excess ammonium nitrate was applied to one-year-old trees in 2007 and to two-year-olds in 2008. This rate was suspected insufficient for the growth of older reference trees hence a treatment with 60 kg N ha^{-1} was included in 2008. With ^{15}NET, the proportion of atmospheric N_2 (%Ndfa) of *E. angustifolia* in 2007 was 79% when referenced against *U. pumila* and 68% against *G. triacanthos.* The results of the AV method showed that the %Ndfa of two-year-old *E. angustifolia* was 80%, and 68% when referenced against the same two species, respectively. Over two years, *E. angustifolia* fixed an average of 16 kg N_2 ha^{-1} $year^{-1}$ when compared against both reference species. The findings of the N_2 fixation rates of *E. angustifolia* measured with the ^{15}NET and AV methods were compared also with the ^{15}N natural abundance (^{15}NA) and total N difference (ND) methods. The highest accuracy was obtained with the AV method, but financial and material considerations may favor the total ND method, especially when used in lysimeter trials when facilities are accessible for accurate dry matter and total N determination.

Tree plantation management would benefit from tools to support harvest scheduling of *E. angustifolia* and *R. pseudoacacia* foliage that have high N contents. During 2006-2008, the chlorophyll meter SPAD-502 was tested, calibrated and validated in the core experiment for the N_2-fixers and the non-N-fixer *G. triacanthos*. The temporally and spatially based validation of the species showed very high correlations with the empirically monitored values. The SPAD-502 is therefore helpful for livestock rearers who intend to include tree foliage in feed diets of their animals. Leaf crude protein (CP), an important indicator for feed quality, can now be determined for the three species given the established relationships between N/CP and the SPAD-502 readings. This determination, however, is only valid within the SPAD-502 range of readings determined during the calibration process for each species. Based on a least-cost-ratio model, the time of inclusion of foliage in the feed was simulated, which predicted that the leaves of non-fixer *G. triacanthos* would be best harvested in May, whereas the N_2-fixer *E. angustifolia* should be harvested in July and September. Therefore, P fertilization at planting and the use of optical-based sensors during the vegetation season are two potential means for improving tree plantation management on marginal croplands that benefit the environment and farmers in the dryland regions of Uzbekistan.

Auswirkungen von Phosphorzugaben auf die Stickstoffbindung und das Wachstum von Bäumen auf Agrarflächen mit hoher Bodensalinität im Unterlauf des Amu Darya in Usbekistan

KURZFASSUNG

Eine Aufforstung speziell mit N_2-bindenden Bäumen stellt eine Möglichkeit zur Restaurierung von salzbefallenen Agrarflächen am Unterlauf des Amu Darya dar. Die Bindungsraten von N_2 hinsichtlich eines verbesserten Wachstums junger Baumbestände in der Bewässerungslandschaft von Khorezm in Usbekistan sind jedoch kaum untersucht. Ein besseres Verständnis darüber kann die Bodenfruchtbarkeit und somit die Produktivität solcher degradierten Flächen in der Region steigern, und damit letztendlich das Einkommen der Bauern erhöhen. Ein Zweifaktorenversuch, durchgeführt zwischen 2006-2008, diente daher der Untersuchung der Auswirkungen von drei unterschiedlichen Phosphorzugaben (P) auf N_2-Bindung, Biomasse und Wachstumsraten der Aktinorrhiza-Pflanze *Elaeagnus angustifolia* L. und der Leguminose *Robinia pseudoacacia* L. Die Bindung von N_2 wurde dabei anhand der natürlichen ^{15}N Häufigkeit bestimmt, auf Blatt- und Baumebene im Vergleich mit der nichtbindenden Art *Gleditsia triacanthos* L.. Die Phosphorgaben wie folgt dosiert: (i) hohe Zugaben (90 kg P ha^{-1}), (ii) niedrige Zugaben (45 kg ha^{-1}) und (iii) keine Zugabe (0-P). Im Vergleich mit 0-P stieg die Bindung von N_2 unter hoher P-Zugabe bei *E. angustifolia* um 81%, bei *R. pseudoacacia* sogar um fast das Doppelte. Bei einer Bestandsdichte von 5 714 Bäumen pro Hektar stieg die N_2-Bindung innerhalb von drei Jahren bei *E. angustifolia* von anfänglich 64 kg ha^{-1} auf 807 kg ha^{-1} N_2, und bei *R. pseudoacacia* von 9 kg ha^{-1} auf 155 kg ha^{-1}. Der Effekt der Phosphorgaben auf Wachstum und Biomassezunahme der Bäume zeigte jedoch Widersprüche. Verglichen mit 0-P resultierte eine hohe P-Beigabe in einer Zunahme der absoluten Biomasse, der oberirdischen Biomasse und der Biomasse der verschiedener Baumabschnitte, jedoch waren die Effekte statistisch nicht signifikant. Demgegenüber ergab eine hohe P-Beigabe signifikant höhere relative Höhenwachstumsraten für *E. angustifolia* und höhere Zuwachsraten (Unit Production Rate) als auch Stickstoffproduktivität für *R. pseudoacacia*. Wachstumsanalysen von Bäumen sollten daher sowohl absolutes als auch relatives Wachstum berücksichtigen, um einen vollständigen Einblick zu gewährleisten.

Die N_2-Bindung durch *E. angustifolia*, der vielversprechendsten Spezies im Feldversuch, wurde zusätzlich in Lysimeterexperimenten untersucht. Hierbei wurden mehrere Referenzarten und unterschiedliche Quantifizierungsmethoden verglichen, um möglichen Ungenauigkeiten auszuschließen, die bei der Ernte von großen Baumbeständen im Feld auftreten können. Die Quantifizierungsmethoden umfassten die ^{15}N-Anreicherungstechnik (^{15}NET) und den A-Wert (AW). Die nicht Stickstoff fixierenden Spezies *G. triacanthos* und *Ulmus pumila* dienten dabei als Referenz. Im Jahr 2007 wurde den ein- und 2008 den zweijährigen Bäumen je 20 kg ha^{-1} von mit fünf Atomprozent ^{15}NET angereichertem Ammoniumnitrat zugegeben. Diese Menge war für das Wachstum von älteren Referenzbäumen nicht ausreichend, weswegen 2008 hier 60 kg ha^{-1} Stickstoff zugegeben wurden. Die ^{15}NET Methode erbrachte im Jahr 2007 für *E. angustifolia* verglichen mit den Referenzpflanzen *U. pumila* und *G. triacanthos* einen Anteil an atmosphärischem N_2 (%Ndfa) von 79% bzw. 68%. Die Ergebnisse der AW Methode bei zweijährigen *E. angustifolia* (2008) zeigten einen %Ndfa von 80% und 68% verglichen mit den genannten Referenzarten. Über zwei Jahre hinweg wurden im Vergleich zu beiden Referenzarten durch *E. angustifolia* im Mittel 16 kg ha^{-1} N_2 gebunden. Die durch die ^{15}NET und AW Methoden gemessenen N_2-Bindungsraten von *E. angustifolia* wurden auch hinsichtlich der natürlichen Häufigkeit von ^{15}N (^{15}NA) und durch die Berechnung von Gesamtstickstoff-Differenzen (ND) ausgewertet. Die höchste Genauigkeit wurde hierbei mit der AW Methode erzielt. Die ND Methode ist hingegen kostengünstiger und

insbesondere in Lysimeterexperimenten zu bevorzugen, wenn auf eine gute Infrastruktur zur Bestimmung der Trockenmasse und des Gesamtstickstoffs zurückgegriffen werden kann.

Werkzeuge zur Planung von günstigen Erntezeitpunkten, also den Zeiträumen mit hohen Stickstoffgehalten der untersuchten Arten *E. angustifolia* und *R. pseudoacacia,* können das Management von Baumplantagen verbessern. Zwischen 2006 und 2008 wurde das Chlorophyll-Messgerät SPAD-502 im Hauptexperiment mit den beiden N_2-bindenden sowie der nicht N_2-bindenden Baumart *G. triacanthos* getestet, kalibriert und validiert. Die räumliche und zeitliche Validierung der Baumarten zeigte hohe Korrelationen mit empirisch gewonnenen Werten. Zusätzlich wurden für die drei Baumarten Bandbreiten von SPAD-502 Messungen ermittelt, innerhalb derer eine Überwachung und Vorhersage der zeitlichen Entwicklung von Stickstoffgehalten der Blätter mittels der statistischen Zusammenhänge möglich ist. Dieses Gerät kann tierhaltende Betriebe unterstützen, die Baumlaub optimal in die Zufütterung ihres Tierbestands integrieren möchten. Für die Bestimmung des Futterwerts der Blätter ist der Gehalt an Rohprotein (RP) von besonderer Bedeutung. SPAD-502 erlaubte die Bestimmung des RP-Gehalts von Blättern der drei Baumarten auf Grundlage des bekannten Verhältnisses von Stickstoff zu Rohprotein (N/RP), allerdings nur im Bereich der mit dem SPAD-502 bei der Kalibrierung für jede Art festgelegten Meßwerte. Die minimale Kostenkombination verschiedener Intensitäten von Blattfütterung während der gesamten Vegetationsperiode wurde mit einem gesondert entwickelten Rechenmodell simuliert. Wesentliche Ergebnisse waren, dass die Blätter des nicht-stickstoffbindenden *G. triacanthos* am Besten im Mai geerntet werden sollten, wogegen der optimal Erntezeitpunkt des stickstoffbindenden *E. angustifolia* zwischen Juli und August liegt. Die Düngung von Phosphor bei der Baumpflanzung und die Nutzung optischer Sensoren sind daher sehr gut geeignet, das Baumplantagenmanagement auf marginalen landwirtschaftlichen Flächen zu verbessern, was der Umwelt und den Farmern in den Trockenregionen von Usbekistan zu Gute kommt.

РЕЗЮМЕ

Лесоразведение, в особенности с использованием азотофиксирующих пород, является одним из вариантов улучшения экологического состояния засолённых орошаемых земель в низовьях Амударьи. Однако показатели роста молодых деревьев и уровень фиксации ими атмосферного азота на маргинальных землях Хорезмской области Узбекистана изучены недостаточно. Исследование данных вопросов может способствовать повышению продуктивности таких земель, улучшению их плодородия, а также увеличению доходов фермеров.

Исходя из этого, в 2006-2008 гг. был проведён двухфакторный полевой опыт для определения эффективности норм фосфорных удобрений на размеры азотофиксации, накопление биомассы и темпы роста азотофиксирующих пород, таких как *Elaeagnus angustifolia* L. и *Robinia pseudoacacia* L. Фиксация атмосферного азота была определена методом натурального обогащения ^{15}N (^{15}NA) как в образцах листьев, так и целого дерева. В качестве контроля была использована *Gleditsia triacanthos* L., как порода дерева, нефиксирующая азот. Нормы фосфорных удобрений составили: (i) 90 кг/га (высокая норма), (ii) 45 кг/га (низкая норма), и (iii) контроль (без внесения фосфора).

Результаты исследований показали, что при внесении высокой нормы фосфора фиксация азота породой *E. angustifolia* повысилась на 81% и почти удвоилась у породы *R. pseudoacacia* по сравнению с контролем без фосфора. К концу 3 года исследований, размер фиксации азота породой *E. angustifolia* при внесении высокой нормы фосфора увеличился с 64 до 807 кг/га, и с 9 до 155 кг/га у породы *R. pseudoacacia* при густоте стояния деревьев 5714 штук/га. Действие фосфорных удобрений на увеличение биомассы и абсолютный рост деревьев имело непостоянный характер. По сравнению с контрольным вариантом, внесение высокой нормы фосфора повысило общую биомассу, общую надземную биомассу и биомассу различных фракций, однако увеличение абсолютного роста деревьев было статистически недостоверным. В отличие от этого, внесение высокой нормы фосфора повысило относительные темпы роста в высоту у *E. angustifolia*, а также темпы производства биомассы с учётом многолетних фракций и продуктивность азота у *R. pseudoacacia*. Следовательно, для полной оценки продуктивности древесных пород необходимо изучать показатели как абсолютного, так и относительного темпов роста деревьев.

Фиксация атмосферного азота породой *E. angustifolia*, которая показала высокий потенциал в полевом эксперименте, была также определена в лизиметрических установках, т.е. в контролируемых условиях, с целью исключения всевозможных отклонений, которые могут иметь место при сборе биомассы с больших деревьев на открытых участках. В данном эксперименте в качестве контроля использовались древесные породы *G. triacanthos* и *Ulmus pumila* L. и применены два метода определения азотофиксации: (1) метод изотопного разведения (^{15}NET), когда вносится одинаковая норма N-удобрения под фиксирующие и нефиксирующие атмосферный азот древесные породы, (2) А-метод (AV), когда вносятся разные нормы N-удобрения под фиксирующие и нефиксирующие атмосферный азот древесные породы. В 2007 г. азот в норме 20 кг/га и в форме нитрата аммония с обогащением изотопа азота ^{15}N 5 ат. % был внесен под однолетние деревья, а в 2008 г. - под двухлетние деревья. Предполагалось, что норма 20 кг N/га является недостаточной для нормального роста двухлетних контрольных пород. Поэтому в 2008 г. в эксперимент был введен дополнительный вариант с нормой азота 60 кг/га. При использовании метода ^{15}NET фиксация азота породой *E. angustifolia* в 2007 году составила 79% в сравнении с контрольной породой *U. pumila,* и 68% в сравнении *G. triacanthos*. Результаты метода AV показали, что процент фиксации азота двухлетней *E. angustifolia* составил соответственно 80% и 68% в сравнении с теми же контрольными древесными породами. Размер фиксации атмосферного азота породой *E. angustifolia* в среднем за два года изысканий составил 16 кг/га относительно упомянутых контрольных древесных пород. Размеры азотофиксации, определенные двумя методами изотопного разведения, были сопоставлены с результатами метода натурального обогащения ^{15}N и методом «разности» по выносу общего азота между неудобренным и удобренным вариантами опыта. Выявлено, что в данном случае А-метод является наиболее

точным. Однако если учесть финансовые и материальные стороны, метод «разности» был признан более подходящим, особенно при его использовании в условиях лизиметрических установок, где существуют возможности для более точного определения сухой массы деревьев и общего азота в растительных образцах.

Использование специального оборудования для разработки графика, который показывает время максимальной концентрации азота в листьях *E. angustifolia* и *R. pseudoacacia* для их сбора в качестве кормовой добавки скоту, принесло бы пользу в управлении древесными плантациями. В этих целях, в течение 2006-2008 гг., был протестирован и откалиброван хлорофиллметр SPAD-502. Правильность калибровки данного прибора затем была подтверждена в полевом эксперименте с двумя азотофиксирующими и одной нефиксирующей азот породами. Проверка полученных в опыте данных во времени и пространстве показала высокую корреляцию между фактическими и смоделированными значениями. Таким образом, хлорофиллметр SPAD-502 является полезным инструментом для животноводов, которые намерены ввести древесную листву в качестве добавки к основному корму. Более того, благодаря установленной зависимости между азотом/сырым протеином и значениями SPAD-502, стало возможным определение содержания сырого протеина в листьях трёх пород как основного индикатора качества корма. Однако эти данные приемлемы в случае, если показания SPAD-502 находятся в пределах значений, определённых при калибровке прибора для каждой древесной породы. С помощью модели линейного программирования были определены оптимальные сроки сбора лиственного корма: у *G. triacanthos* в мае, а у *E. angustifolia* – в июле и сентябре. Следовательно, внесение фосфорных удобрений при посадке древесных пород и использование оптических сенсоров, таких как SPAD-502, в течение вегетационного периода могут способствовать улучшению управления древесными плантациями на маргинальных землях. Это в свою очередь, окажет благоприятное воздействие на окружающую среду и на уровень жизни населения засушливых регионов Узбекистана.

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LIST OF ACRONYMS AND ABBREVIATIONS

ANOVA	Analysis of variance
BNF	Biological nitrogen fixation
CA	Central Asia
CARs	Central Asian Republics
CIS	Commonwealth of Independent States
CP	Crude protein
CP:ME	Ratio of crude protein to metabolizable energy
DM	Dry matter
dO	Digestibility of organic matter
EC	Electrical conductivity
FAO	Food and Agricultural Organization
Gb	Gas production
GOU	Government of Uzbekistan
GWT	The groundwater table
LP	Linear programming
LS means	Least square means
ME	Metabolizable energy
MJ	Mega Joule
N	Nitrogen
NFTs	N_2-fixing trees
NP	Nitrogen productivity
P	Phosphorus
PEG	Polyethylengglycol
RGR	The relative growth rate
RGR_D	Relative diameter growth rate
RGR_H	Relative height growth rate
RMSE	Root mean squared error
RRMSE	Relative root mean squared error
RSR	Root to shoot ratio
SPAD	Soil Plant Analyses Development
SSP	Super simple phosphate
UPR	Unit production rate
ZEF	Zentrum für Entwicklungsforschung (Center for development research)

1 GENERAL INTRODUCTION

There are many reasons for planting, maintaining and managing trees in a landscape. These vary from ecological service provision (maintaining air quality, climate amelioration, water conservation, soil preservation, wildlife support and many others) to social roles, given the place of trees in religion, art, history and politics in communities, and also for improving livelihoods, given the many tangible and intangible benefits of trees. The role trees can play as a natural capital in the pathways to food security, poverty eradication and sustainable development has been underpinned by the United Nations (UN) in their Millennium Development goals (UN Millennium Project, 2005) and in the Framework Convention on Climate Change (1992). Much of the matters spelled out in these manuscripts should be of interest to decision makers, administrators and land users in Central Asia when planting, maintaining and managing trees on the marginal croplands in the irrigated landscapes.

During the 20th century, the forest and woodland areas in Central Asia declined by on average 4-5 times due to an increasing anthropogenic impact (FAO, 2006a). The present forest areas account for less than 7.3% of the entire region, but this is unevenly distributed over the five countries Kazakhstan, Kyrgyzstan, Tajikistan, Turkmenistan, and Uzbekistan (FAO, 2006a). During the Soviet era (1924-1991), forests and woodlands were promoted in Uzbekistan for environmental services provision, for combating land degradation and desertification (Tupitsa, 2010) and for protecting watersheds and conserving bio-diversity (Vildanova, 2006). Following independence in 1991, the government of Uzbekistan (GOU) included tree plantings on their development agenda, but this was mainly for satisfying the domestic demand for fruits and grapes (GOU, 2006b), construction wood and pulp (GOU, 1994) and for re-vitalizing sericulture (GOU, 2006a). In the National Action Plan of 1999, the role of trees in landscape restructuring was outlined, although it remains unclear if this plan was eventually accepted. The year 2011 was declared by the UN assembly as the International Year of Forests to raise awareness on the role of trees and forests in reaching conservation and sustainable development. In pursuit of this, the GOU decided on a nationwide launch to plant trees in this year (Figure 1.1).

Figure 1.1: Banner installed in the center of Urgench city by the local Khokimiyat in accordance with the declaration of the year 2011 as the International Year of Forests. March, 2011.
Translation: Dear compatriots: Let us take an active part in planting fruit and ornamental trees under the slogan: "For the 20th anniversary of independence my gift is 20 trees"

1.1 Problem setting

Prior to becoming socialistic republics within the Soviet Union (SU) bloc in 1924, the five Central Asian[1] Republics (CARs) Kazakhstan, Kyrgyzstan, Tajikistan, Turkmenistan, and Uzbekistan had a Turkic-Persian history in common as evidenced by their similar linguistic roots, various cultural and social habits and the conviction to the Islam religion. For most of the 20th century, the CARs had been united in the centrally managed, single political, economic and infrastructure system of the SU (Suleimenov, 2000). Final directions and decisions, e.g., on agricultural production, were taken by the leaders of the Communist Party, not only of the SU, but also by the leadership of the republics, the region and districts (Suleimenov, 2000). To increase both overall agricultural production and the arable area, the agricultural sector in the CARs was modernized. This was motivated by the unprecedented view that not the irrigable land was limiting for the development of the agricultural sector of the SU, but rather the amount of irrigation water that needed to be diverted to unexploited areas (Field, 1954). The well-known outcome of this vision of modernization was a centrally planned

[1] Central Asia is also referred to as Middle Asia by Russian geographers

system of mono-cropping over large areas, and that each of the CARs became specialized in the production of one strategic commodity as one component of the larger system. Uzbekistan, for example, was consigned to produce cotton (*Gossypium hirsutum* L.) with energy gulping production systems and supported by high inputs from the state that also procured the commodities produced. This historic decision also explains the different development paths of the agricultural sectors of the CARs. Not only did these already begin during the Soviet era, they also indirectly were the signal and set the framework for the development paths pursued after independence in 1991 (Suleimenov, 2000).

After independence, the five CARs agreed to become part of the Commonwealth of Independent States (CIS). Although each republic pursued different development paths, land reforms initiated private land ownership in all CARs, albeit at different speed and to different degrees (Spoor and Visser, 2001). Owing to secondary soil salinization, water logging and saline shallow groundwater tables, the new land users, cultivating about 8 Mha of irrigated lowland areas of the CARs of which 50% lies in Uzbekistan, struggled to cope with the inherited soil degradation and with rising water demands and at the same time declining supply of water (Spoor, 1999). The tragic consequences of an inadequate modernization of the agricultural sector can be seen in the Khorezm region in northwest Uzbekistan, which is representative of the irrigated lowlands in the CARs. It is for this reason that the concept of ZEF (Center for Development Research, Bonn, Germany) for sustainable resource use envisages alternative uses of marginal cropland in the Khorezm region such as afforestation, rather than further promoting an over-exploitation of such croplands with, for example, annual crops. Trees play a pivotal role in ZEF's concept of restructuring of land and water resources at the farm and landscape level (Martius et al., 2004).

Once the appropriate tree species are identified, afforestation is an effective remedy to re-vegetate saline landscapes, reduce elevated groundwater tables and mitigate dryland salinization in irrigated land-use systems (Marcar and Crawford, 2004). Therefore, indigenous tree species in the Khorezm region were screened for their ability to survive on drought-prone and saline sites, i.e., in resource-poor environments (Khamzina et al., 2006). The indicators included (1) survival rates, (2) ability to rapidly develop belowground biomass to assure early development, (3) aboveground biomass increment, (4) rate of growth in height and trunk diameter, and (5) potential for use as supplementary fodder, fuelwood and construction material. A ranking of species considering all criteria simultaneously pointed at the high potential of *Elaeagnus angustifolia* L. to afforest marginal croplands. Although the performance of the other species differed by soil type and criteria considered, the overall assessment also indicated the potential for afforestation with *Ulmus pumila* L. and *Populus eupharatica* Olivier (Khamzina et al., 2006).

When assessing the potential of trees for afforestation of degraded landscapes in the irrigated lowlands of Central Asia, an understanding of the relationship between tree species and two key ecosystem functions is compulsory, i.e., the water and nutrient cycles (Patabendige et al., 1992; Bell, 1999; Marcar and Crawford, 2004). Previous studies in the Khorezm region addressing the water cycle examined the transpiration capacity and water use of nine 2-4 year old tree species, which were used as a proxy for their potential for bio-drainage and enhancing groundwater discharge (Khamzina et al., 2006; Khamzina et al., 2009b). Owing to differences in physiological features of the species and water uptake by roots, the average daily leaf transpiration varied from 4.5–5.2 mmol m^{-2} s^{-1} in the case of *Prunus armeniaca* L. to a peak of 4.5–10 mmol m^{-2} s^{-1} for *E. angustifolia* (Khamzina, 2006). In particular *E. angustifolia*, *U. pumila*, *P. euphratica* and *P. nigra* var. *pyramidalis* showed a high bio-drainage potential given an average annual stand transpiration that varied between 1250 mm (*E. angustifolia)* and 670 mm (*U. pumila*), whilst fruit species such as *P. armeniaca* and *Morus alba* L., showed low bio-drainage potential. The transpiration of tree stands led to a daily cycle of the groundwater. During the day, significant reductions in groundwater level were monitored but these did not last. During the night, the groundwater used to rise again. Hence, a permanent lowering of the groundwater level did not occur owing to the recharge of the groundwater body caused by the continuous irrigation of the irrigated croplands in the vicinity of the afforested site (Khamzina et al., 2009b).

The role of trees in nutrient (re)cycling is multiple. Trees serve as a nutrient pump taking up minerals from deeper soil layers and bringing them to the soil surface when shedding leaves. During the decay process, the released nutrients can contribute to maintaining and improving soil fertility. A comparison of the potential of *E. angustifolia*, *U. pumila* and *P. euphratica* to supplement via leaf decay the plant-soil nitrogen (N) stocks in the Khorezm region (Lamers et al., 2010) showed that *P. euphratica* foliage decomposed fastest with a 61% weight loss after one year, whilst *E. angustifolia* and *U. pumila* leaves showed weight losses of 51% and 52%, respectively. Yet, despite a lower foliage decay rate, *E. angustifolia* had the highest potential for soil bio-amelioration owing to the combination of high N-leaf concentrations and the highest foliage production of ca. 6 t ha^{-1}, which was almost threefold higher compared to the other species. The contribution to the soil-plant system by *E. angustifolia* was estimated at 97 kg N ha^{-1}, compared to 33 kg N ha^{-1} by *U. pumila* and 23 kg N ha^{-1} by *P. euphratica*.

Afforestation with (fast-growing) N_2-fixing tree species (NFTs) is a preferred option for ecological restoration of highly salinized irrigated croplands (Brewbaker, 1989; Peoples and Crasswell, 1992). N_2-fixation can make a major contribution to sustainable agriculture by maintaining soil fertility, but information about the N_2-fixing capability of trees on saline soils is sparse. Following a review, Danso et al. (1992) concluded that N_2-fixing trees (NFTs) may capture as much as 43-581 kg of N ha^{-1} annually. The authors also reported N_2-fixation rates by 4-year-old *Robinia pseudoacacia* stands as high as 220 kg N ha^{-1} when measured by the ^{15}N isotope dilution method (Danso et al., 1995). Another review estimated an annual fixation of 112 kg N ha^{-1} $year^{-1}$ measured in 25-year-old *R. pseudoacacia* stands using the acetylene reduction assay (Noh et al., 2010). Based on the ^{15}N natural abundance method, actinorhizal *E. angustifolia* in the Khorezm region annually fixed 24-514 kg N ha^{-1} (depending on the age of the tree stands), which seemed to be sufficient to satisfy crop-N demand on the salt-affected croplands and hence to maintain growth (Khamzina et al., 2009a). Despite the accumulated body of evidence, including a wide range of bio-physical (Khamzina et al., 2006) and socio-economic (Kan et al., 2008; Lamers et al., 2008) indicators examined for assessing the potential of N_2-fixing woody species for afforestation and for providing wooden and non-wooden benefits, options to boost the establishment and juvenile growth of actinorhizal *E. angustifolia* with the least costs have not been studied. Yet this is of interest for increasing ecological service provision and income generation of land users. Furthermore, aside from the *Frankia*-non-legume symbioses of *E. angustifolia*, other associations were not included, although for instance *Robinia pseudoacacia* L. (Black locust), which is a representative of a woody-legume-

Rhizobium symbiosis, is a widespread woody legume in arid Central Asia, and is in fact one of the three most widely planted broadleaf tree species worldwide based on the total hectares established (Keresztesi, 1980).

To boost early growth and enhance tree stand establishment of the N_2-fixing legume (*Rhizobium*-legume symbiosis) and non-legume (*Frankia*-nonlegume symbiosis) tree species, various recommendations are made, which however all have both advantages and disadvantages. Nitrogen fertilization, for instance, effectively enhances early growth, but the use of N is not only expensive but also represses N_2-fixation (Fried and Broeshart, 1975). N_2-fixation rates and growth of perennial plants can also be increased through phosphorus (P) amendments. Although this was seen to be highly effective in tropical regions (Balasubramanian and Joshaline, 1996; Wheeler et al., 1996; Sanginga, 2003), information on its impact is deficient for legumes and non-legume woody species grown on the impoverished and saline soils of Central Asia.

Variations in tree N_2-fixation are associated not only with the species, soil conditions, age and density of plantations, but also with difficulties related to a complete, labor-demanding harvesting of large mature trees (Boddey et al., 1995). Such inconveniences can be avoided by the use of lysimeters. In this way, reliable estimates can be gained while assessing N_2 fixation based on whole trees. Accurate quantification of N_2 fixation by woody species has not yet been undertaken in Central Asia although this knowledge is vital for setting up experiments, especially because the use of enriched ^{15}N fertilizers for assessing N_2 is resource demanding.

Another cause for the reportedly varying amounts of N_2 fixation are the methods used for quantifying biological nitrogen fixation (BNF) by trees (Boddey et al., 1995), which all have certain limitations (Peoples et al., 2002). The choice of the correct method is key for accurate N_2 quantification, which can be achieved also when using a combination of methods and the concurrent use of multiple reference species (Boddey et al., 1995). Yet, no direct comparison exists of the different methods for quantifying N_2 fixation by species home to Central Asia such as actinorhizal *E. angustifolia*, on which relatively few studies have been focused thus far, or leguminous *R. pseudoacacia,* on which hardly any research results could be found for the Central Asia region.

Irrespective of the motivation to plant and maintain trees in a landscape, a judicious management is both beneficial and compulsory. Although the type of management depends, among others, on the purpose of the plantations and the trees in the landscape, at present hardly any decision-support tools exist. Given that leaf-N concentrations are an appropriate indicator for improving management and optimizing cultivation practices of annual crops, various *in-situ*, non-destructive, diagnostic tools have been developed including the optical sensor SPAD (Soil Plant Analyses Development)-502 chlorophyll meter (Minolta, 1989). Despite their proven efficiencies in a wealth of studies (Peng et al., 1993; Balasubramanian et al., 2000; Singh et al., 2002), their use demands *a priori* calibration, as the relationships between readings and leaf-N content are vegetation and species specific (Peterson et al., 1993). In addition, since leaf vegetation contains different N concentrations, which consequently give different SPAD-502 indices, the range of the SPAD-502 readings that accurately predict leaf N needs to be determined. Such relationships and calibrations are deficient not only for tree species in general (Moreau et al., 2004), but also especially for the species predominant in the landscapes of Central Asia. Once reliable relationships are identified between for instance the SPAD-502 readings and the tree leaf N status and these are calibrated, they can provide great support in tree and tree plantation management, e.g., selection of appropriate species, leaves and time of leaf consumption for improving and enriching feed diets.

Tree leaves contain N, which is a basic component of protein (Menke et al., 1979). Therefore, tree foliage is used worldwide for complementing feed diets (Devendra, 1992; Reddy and Elanchezhian, 2008). The N/crude protein-rich tree foliage of certain tree species also has the potential for improving the diets and health of, for instance, cattle in Khorezm (Lamers and Khamzina, 2010), where feed demand is outstripping the traditional production of wheat bran, maize, sorghum, or rice stover. These feedstuffs have, however, low nutritional value (Lamers and Khamzina, 2010), which is a major cause for the present low average milk production (6-7 l per day per cow) (Djanibekov, 2008). Fast-growing *E. angustifolia* possessed, for example, multiple benefits including nutritional leaves. Previous research in the Khorezm region, however, only provided information on the use of tree foliage *per se* and the most promising tree species (Lamers and Khamzina, 2010). The optimal timing for collecting tree leaves to complement dairy diets while at the same time considering nutritive and financial aspects has not yet been addressed. The effect of P fertilization on foliage N content and feed quality is unknown either. Hence, knowledge on the N/crude protein dynamics of tree foliage over the growing season that could be provided for by optical sensors can help to determine the optimum harvesting time of tree leaves and the use of tree foliage at low costs for livestock holders.

1.2 Research objectives

To bridge the present gaps in the establishment, production and management of suggested tree plantings, specifically of N_2-fixing tree species such as the actinorhizal *E. angustifolia* and the leguminous *R. pseudoacacia* in a resource-poor environment caused by soil salinization, the overarching objectives of this study were to:

(1) Assess the effect of P applications on boosting juvenile growth, N_2 fixation rates and foliage N content of N_2-fixing tree species based on the morphological and physiological characteristics of the trees using the indicators total biomass production, growth rates, N productivity, foliage N content and the contribution of N_2 fixation by these species to the soil-plant system;

(2) Determine the N_2 fixation rates of actinorhizal *E. angustifolia* in lysimeters to obtain accurate whole-tree estimations, and appraise four methods of measuring N_2 fixation;

(3) Analyze the suitability of the chlorophyll meter SPAD-502 for monitoring the leaf-N content of three tree species and generate a calibration dataset for predicting leaf-N dynamics;

(4) Determine the optimum harvesting time and the best admixture of N/crude protein-rich tree leaves and common feedstuff for improving and enriching feed diets.

1.3 Structure and outline of the thesis

This thesis consists of eight chapters. Following this chapter with a general introduction to the problem setting and the state-of-the-art knowledge, the geographical, agro-climatic and other key characteristics of the Khorezm region are described in Chapter 2. The growth of two N_2-fixing trees and their N_2 fixation rates as influenced by additions of different P levels in an open field trial are analyzed and discussed in Chapter 3. In the following two chapters, the results of the quantification of N_2 fixation by *E. angustifolia* against two reference trees in a lysimeter trial are presented. While focusing on the ^{15}N

isotope dilution method (Chapter 4), a direct comparison with the outcomes of the field experiments is made (Chapter 3). Secondly, as part of the appraisal of four methods for measuring N_2 fixation, in particular the accuracy and need of financial resources were compared (Chapter 5). The focus in Chapter 6 is on the use of the chlorophyll meter SPAD-502 for quick determination of leaf-N content of three tree species, the calibration of the SPAD-502 readings for these species, and the elaboration of calibration equations for each of these species for predicting their leaf-N dynamics. Using a linear programming model, the application of these results allowed determining the optimum harvesting time of tree foliage and optimization of a feed mix based on the various common feeds (Chapter 7). Each of the Chapters 3-7 starts with a brief introduction, followed by the methodology used, the results and their discussion and conclusions. In Chapter 8, an overall discussion and conclusions and recommendations are presented followed by an outlook for the future.

2 STUDY REGION

2.1 Geographical setting and demography

Khorezm was already reported upon in documents on ancient and medieval times in Central Asia. The region used to cover a territory that today comprises Uzbekistan and Turkmenistan. Khorezm currently covers 605,000 ha in the northwest of Uzbekistan, and is located between 41.13°-42.02°N latitude, i.e., on the same latitude as cities such as Marseille and New York, and 60.05°-61.39°E longitude (Figure 2.1).

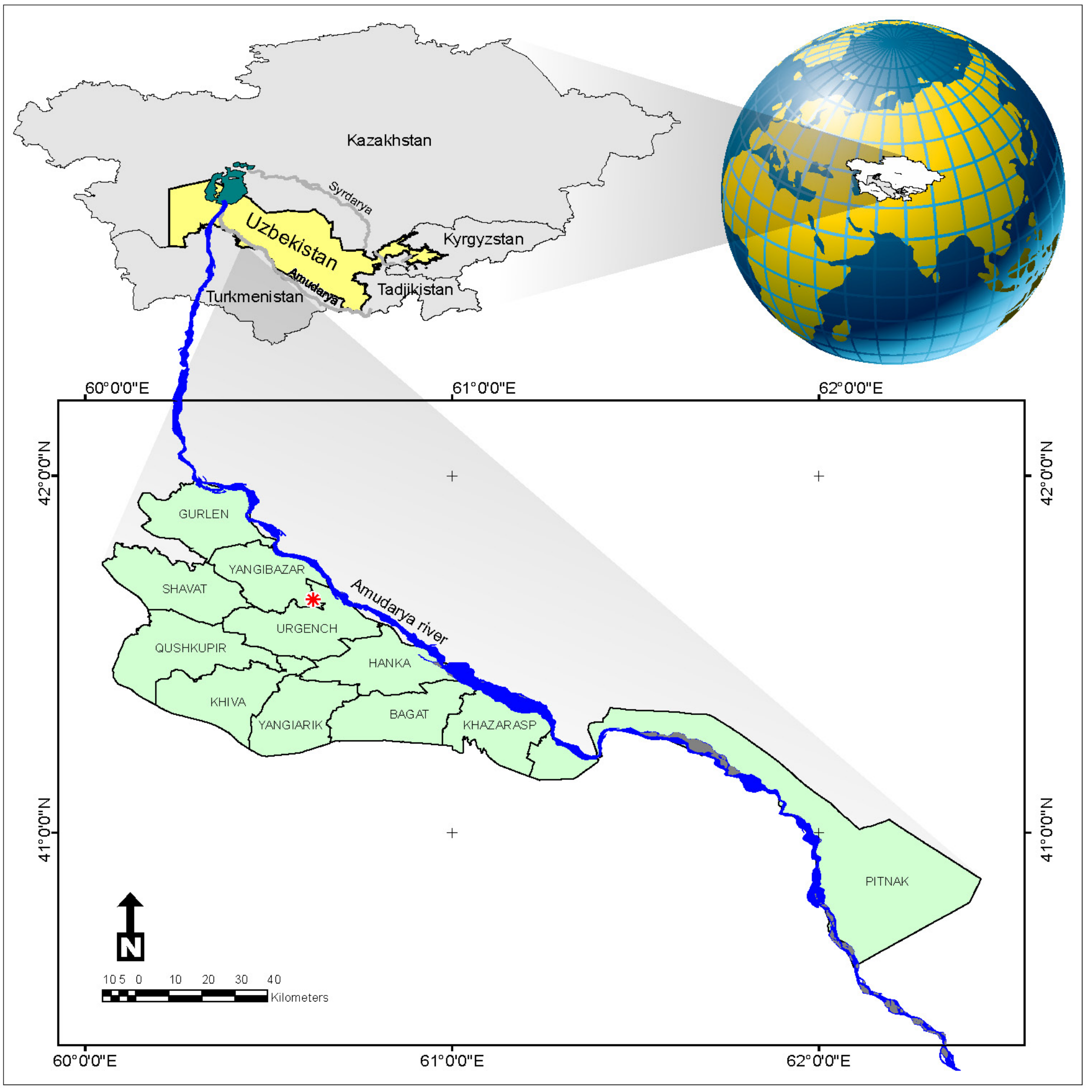

Figure 2.1: Location of the study region, the administrative sub-districts (green color) and experimental site (red dot)

With reference to the Baltic Sea, the flat topography of the Khorezm region ranges between 75 and 138 m a.s.l. The region is situated on the lower reaches of the Amu Darya River and is part of the northern Turan lowlands of Central Asia (Tupitsa, 2010). The distance to the present shores of the Aral Sea is about 260 km to the north (Shanafield et al., 2010), and the Sarykamish depression is 200 km east of the region. The extreme northern point of the region is the Nuronbobo wild forest (Nuronbobo tuqay) near Olchinn village in the administrative district Gurlan, the most southern is the town Tuprakkala in the administrative district Khazarasp (KRK, 2008-2011).

Of about 605,000 ha in Khorezm, about 270,000 ha have been made suitable for irrigated agriculture, which forms the cornerstone of the livelihood security of ca. 1 million rural inhabitants, i.e., about 70% of the total 1.5 million people (as of 1 July 2008). Density is 249 persons km^{-2} (KRK, 2008-2011). Urgench is the capital of the Khorezm region with a population of 135,500 (as of 2008).

2.2 Climate

The Khorezm region is part of the Central Asian semi-desert zone, with a continental climate as evidenced by the large differences in seasonal daily light intensity, day length and temperatures (Table 2.1). Several key parameters of the prevailing climate indicate that the annual average temperature is about 13 ^{o}C, but in the hottest months average temperatures can be as high as 28^{o}C and in the coldest months as low as -14^{o}C. This indicates that tree species must be able to cope with large temperature differences over short periods. The main peculiarities of the region's climate are changeable weather during one and the same day, air dryness, and (rare) precipitation.

The total annual precipitation amounts to ca. 100 mm (Table 2.1), which mainly falls during the moist winter months and thus outside the growing season, which is from April till October. The 6-year frost-free period averaged 282 days (Table 2.1). The approximate mean annual temperature of about 13^{0}C, long-term annual precipitation of about 100 (MAP) mm, and potential evapotranspiration (PET) of about 1000 mm gives an aridity indicator of 0.08 (MAP/PET), which indicates an arid bioclimatic zone (Gintzburger et al., 2003).

Table 2.1: Summary of weather and climate parameters of Yangibazar meteorological station (41°65′ N latitude, 60°62′ E longitude, altitude 102 m a.s.l.), 2003-2008

Item	Unit	Mean	Minimum	Maximum	Range	Std. Error
Sum of effective temperatures (T) per year above 10 ^{o}C	^{o}C	4383	3292	4837	1545	230
No. of days with a daily mean T above 10 ^{o}C	Days	203	142	226	84	12
Date of first day with mean daily T above 10 ^{o}C	Date (dd/mm)	14/03	15/02	20/03	na	na
Date of last day with mean daily T above 10 ^{o}C	Date (dd/mm)	11/11	7/11	23/11	na	na
Annual precipitation	mm	113	20	212	193	29
No. of rainy days	Days	46	19	87	68	9
No. of rain-free days	Days	286	194	325	131	20
Amount of daily rainfall	mm	1.4	0.1	66.0	65.9	0.2
Annual temperature	^{o}C	13.9	13.2	15.4	2.2	0.3
Temperature range hottest month of the year	^{o}C	27.3	28.0	25.9	2.1	na
Temperature range coldest month of the year	^{o}C	-5.3	-14.6	-0.3	-14.3	na
No. of frost-free days	Days	282	162	340	178	25
Annual relative air humidity	%	61.4	57.4	65.3	7.8	1.3
Annual relative air humidity of those days with a mean annual day temperature > 10 ^{o}C	%	52.6	46.8	55.8	9.0	1.4

na – not available

2.3 Soils

The soils of the Khorezm region are of alluvial origin, and were formed under the influence of the meandering Amu Darya River (Ibrakhimov, 2005) as evidenced also by the ancient beds that can be identified and traced by satellite images and the archaeological sites in remote desert areas. The age-long impact of this river had led to heterogeneously stratified soils throughout the region. However, according to Ibrakhimov (2005), the upper soil layer generally has a silt and sandy loam texture and depths ranging from 2-3 m underlain by sand. According to FAO classification, the soils are: 1) arenosols, gleyic and calcaric (sodic), 2) arenosol and aridic, 3) cambisol and calcaric, 4) fluvisol, gleyic, and humis, and 5) solonchak, takyric and arenosols (Ibrakhimov, 2005). Egamberdiev (2007) characterized the natural fertility of the soils in Khorezm as low due to low soil organic matter (0.7-1.5 g 100 g^{-1}), total nitrogen (N) (0.07-0.15%), phosphorus (P) (0.10-0.18) and available potassium (K) contents.

Soil salinity is the main concern in the irrigated areas of Khorezm. According to official government data (1999-2001), the whole irrigated area shows secondary salinization (Abdullaev, 2003) mainly caused by shallow groundwater tables (GWT), which vary between 0.5 m in the growing season and more than 2 m outside the vegetation period (Ibrakhimov, 2005). Low fertility, increasing soil salinity and elevated GWT are considered unfavorable for the cultivation of most agricultural crops, but selected trees and shrubs that show rapid root development and growth can survive despite these adverse conditions (Khamzina et al., 2006).

2.4 Irrigation and drainage network

An extensive irrigation network with complementary drainage water collectors in the Khorezm Region was constructed between 1950 and 1970 (Sinnott, 1990). The water from the Amu Darya is supplied to the agricultural fields through a complex hierarchy irrigation network consisting of main, inter-farm, and on-farm canals (Ibrakhimov et al., 2004). The most common method of irrigation in the region is furrow (e.g., for cotton cultivation) and flood irrigation (e.g., for wheat and rice production). The drainage system is mainly open horizontal, and all drainage water is directed towards the Sarykamish Depression, which is no longer linked to the Aral Sea (Conrad, 2006).

2.5 Land use

Khorezm is acknowledged as an ancient center of civilization as evidenced by the appearance of the word “Khorezm” in the book “Avesto”, which is one of the rare memorials of Uzbek culture. Reportedly, Khorezm consists of two Persian words – “khvar” – “the sun” and “azm” – the land, the combination of which means “sunny land” (Munis and Agahi, 1999). Even in the distant past, this ancient landscape was known for its favorable climatic and topographic conditions despite the fact that Khorezm has always been surrounded by deserts, i.e., the Karakum to the south and southeast and the Kyzylkum to the east (Conrad, 2006). After water for irrigated crop production had begun to play a major role, agriculture began to bloom, which contributed to the upheaval of the Khiva Khanate people in 1510, due not only to agriculture, but also to trade, brigandage, and slavery (Tolstov, 2005). Irrigation-based agriculture developed during this period centered on the exploitation of small plots (0.3-0.8 ha), which were bordered by earth banks. Trees were planted along the irrigation ditches to reduce evaporation (bio-drainage) (Orlovsky et al., 2000), along fields to protect the soil against wind erosion, and around homesteads as a shield to buffer the extreme weather conditions (Tupitsa, 2010). However, most trees were removed during the Soviet era.

Khorezm became part of the Soviet Union in 1924 (or according to some in 1926) and was consigned for increasing crop production, mainly cotton (*Gossypium hirsutum* L.). Agricultural production was organized through *sovkhozes* and *kolkhozes*. Shortly after Uzbekistan became independent in 1991, numerous land reforms were initiated. Although some authors argue that these reforms mainly ensured a continuation of the previous practices (Veldwisch and Spoor, 2008), the production setup was changed (Djanibekov et al., 2010). Until 2008, the different phases of the land reforms resulted in three main types of farming systems namely: *shirkats* (renamed former state and collective farms), which however were few in number, *fermer* (private farmers), about 19,000 in 2008, and about 240,000 *dekhkan* farms (household plots). These land reforms went hand in hand with reforms in the management of irrigation water from the centralized management during Soviet times to an increased responsibility for water management of the water users (Veldwisch and Spoor, 2008). The number of private farms increased to 242,313 (as of 16 March 2010). Since the land and water reforms did not yield the expected results, the Government of Uzbekistan pursued new land reforms to optimize farm size and increase economic efficiency of the farms. The initiated land consolidation affected both *fermers* and *dekhkan* farms (Djanibekov et al., 2010). From the end of 2008 onwards, for instance, the minimum size of farms[2] defined in the Uzbek legislation was changed for cotton and wheat, gardening and horticulture production units. The minimum size of cotton and wheat farms was increased from 10 ha to 30 ha, while that of horticultural and gardening farms was increased from 1 ha to 5 ha. The

[2] Introduction of Changes and Additions to Legislative Acts of the Republic of Uzbekistan in Connection with Enhancing Economic Reforms in the Agriculture and Water Sectors (2009)

first phase of land consolidation (2008/2009) reduced the number of private farms in Khorezm from 19,000 (before 2008) to around 10,000 by the end of 2008 and to about 5,670 at the onset of 2010. The average farm area thus grew from about 13 ha in 2007 to 24 ha in 2008, and was more than 40 ha in 2010. Through the creation of larger farms, land leasers can be more motivated to withdraw unproductive marginal cropland in favor of afforestation. This withdrawal was less likely to be expected in the case of small-scale farms since these depended on smaller areas for livelihood subsistence (Djanibekov et al., 2010). But farmers with access to larger areas are more likely inclined to improve the production capacities also from marginal land, which comprises some 15-20% of the irrigated area in Khorezm (Conrad, 2006), e.g., through afforestation. The present study was conducted on such a marginal cropland area in the Yangibazar district, where the potential of the soil for cultivating agricultural crops is low and in some places extremely low (Khamzina et al., 2006).

Since the initiation of land reforms, the importance of livestock production for both private and *dekhkan*[3] farms as a source of income and food has grown. However, a concern for the development of livestock production is the feed insufficiency due to the considerable decrease in the area and production of feed crops (Yusupov et al., 2010). The total area under pastures in Khorezm is 71,000 ha. However, within the irrigated area, irrigated pastures only cover 9,400 ha (Yusupov et al., 2010), which is only about 3% of the total irrigated area of Khorezm (ca. 270,000 ha). Given the food demand of the estimated 624,900 cattle including 244,800 cows and 316,600 sheep and goats (Yusupov et al., 2010), this is insufficient to feed such a large number of livestock (Djanibekov, 2006). The feed deficiencies are most prominent during the offseason (November-April), when livestock owners feed their animals at home with wheat and rice straw, maize and sorghum residue and wheat bran (Djanibekov, 2006). Not only the quantity but also the nutritional value of these feedstuffs is insufficient to satisfy the feed needs. Enriched feed would be an option to increase the feed quality, but it is often very expensive and not readily accessible to farmers. Consequently, the feed scarcity is mirrored in, for example, the poor milk production of 6-7 l per day per cow (Yusupov et al., 2010). The introduction of fast-growing, salt-tolerant tree species in the landscape, for example on marginal cropland, can improve both the quantity and quality of fodder

[3] The bulk of livestock output is produced by these small household farms

(Khamzina et al., 2008). However, knowledge is needed on how to optimize the feed ratios with tree foliage.

2.6 Annual and perennial vegetation

The state order has been maintained for strategic crops such as cotton and winter wheat since independence in 1991. Wehrheim and Martius (2008) defined this state order as ”*….a set of dictated and binding rules on crop distribution over the fields, production targets, the supply and price of water and other inputs, product processing and, in the case of cotton, on its export*”. However, the household and garden areas show a wide diversity of annual and perennial crops and species, since these cropping areas are exempted from the state order plans (Bobojonov et al., 2008). On these areas, farmers in the Khorezm region prefer to cultivate fruits and vegetables, which is possible given the warm climate and the extended irrigation infrastructure (Kan et al., 2008). Sericulture was also a widespread practice during Soviet times, and the planting of mulberry (*Morus* spp.) trees was encouraged (Worbes et al., 2006), the leaves of which serve as feed for the silkworm (*Bombyx mori*).

Tree and forest reserves are rare in Khorezm. The present share of 3-4% of the land is more than 50% less than the nationwide average forest cover of 7-10% (FAO, 2006a). Already during Soviet times, the forest reserves were converted into agricultural land for the production of cotton. Since the 1950s, the administration encouraged the re-introduction of tree plantings such as tree windbreaks into the landscape, but design deficiencies limited the anticipated environmental benefits (Tupitsa, 2010). Instead, during the harsh winter months, trees are often illegally taken from the forest and tree reserves (Kan et al., 2008).

More than any other country in Central Asia, except perhaps Turkmenistan, the double land-locked republic of Uzbekistan with a share of 80-90% of total water use in this region relies almost completely on water for irrigation and drinking that originates from outside its own territory (Conrad, 2006). This is critical in particular because of the large area cropped under irrigation (about 4 Mha), the aridity of the climate, and a high population growth and density, particularly in rural areas (ADB, 2006). Since agriculture relies heavily on irrigation, tree plantings may compete for this water source until their root system taps the groundwater for further growth and development (Khamzina et al., 2009b).

2.7 Energy supply and use

Despite the vast fossil fuel reserves exploited in Uzbekistan, a large proportion of the rural population still relies on cotton stalks, cow dung, and particularly firewood for satisfying domestic needs especially during the harsh winter months (Vildanova, 2006). Although logging is considered illegal in Uzbekistan, and licenses are needed for maintenance works of forests and tree reserves, illegal logging in 2004 was estimated to be as high as 380.7 m^3 (Vildanova, 2006).

The conversion of salt-affected cropland to afforested areas could therefore both arrest the presently on-going deforestation of forest reserves (Tupitsa, 2010) and serve as income generation for land users. These could sell high-quality wood from afforestation measures (Lamers et al., 2008), especially because the species recommended for afforestation in the Khorezm region have good firewood characteristics (Lamers and Khamzina, 2008). Reportedly, owing to the general high density and low moisture content of wood of nitrogen-fixing woody species, these species are already potential sources of fuelwood after 3-5 years (Brewbaker, 1989). It should also be noted that in particular the wood from older branches is usually the best component for use as fuel.

Yet, before afforesting and exploiting mixed plantations becomes an acceptable practice, it has been postulated that the existing legal framework needs to be adapted to allow land users to convert marginal croplands into forest areas (Worbes et al., 2006; Kan et al., 2008).

3 EFFECT OF PHOSPHORUS AMENDMENTS ON NITROGEN FIXATION, BIOMASS ACCUMULATION AND GROWTH RATES OF TREES ON SALINE CROPLANDS

3.1 Introduction

Nitrogen (N) is essential for sustaining growth of annual and perennial vegetation, also in the salt-affected, degraded croplands in the arid regions of Central Asia. One option to exploit such croplands more effectively is through afforestation (e.g., Marcar and Crawford, 2004; Khamzina et al., 2006), in particular with N_2-fixing tree species, which have the ability to grow on soils where low levels of available N prevent the growth of other species and vegetation. When integrated into agroecosystems and landscapes, N_2-fixers can support the restoration of nutrient stocks and soil fertility while reducing external inputs such as expensive N fertilizers. In intercropping systems, the sharing of fixed N_2 with interplanted vegetation is known to occur (Van Kessel et al., 1994; Parrotta et al., 1996).

The significance of the woody-legume-*Rhizobium* symbiosis for the rehabilitation of soil in arid regions with high salinity, low fertility and drought periods has been frequently reported (e.g., Brewbaker, 1989; Peoples and Crasswell, 1992). *Rhizobium*-tree legume symbioses, which are able to fix appreciable amounts of N_2 under arid/semiarid and saline conditions, provide a cheap fertilizer substitute and this option has therefore been promoted for land reclamation and landscape improvement in such regions (e.g., Brewbaker, 1989; Peoples and Crasswell, 1992). The role of leguminous woody species in food, feed, fiber, and fuelwood production has also been recognized in a number of studies (Brewbaker, 1989; MacDicken, 1993). For instance, in Australia, trees of the genera *Acacia* provide high-quality animal fodder, fuelwood, charcoal, timber and gums, aside from their contribution to soil improvement (e.g., Brockwell et al., 2005). Yet little is known about the woody-legume-*Rhizobium* symbiosis with tree species in arid Central Asia such as, for instance, *Robinia pseudoacacia* L., a widespread tree species in this region.

Worldwide, actinorhizal[4] interactions (*Frankia*-nonlegume symbioses) contribute the most to N inputs in forests, fields and disturbed sites in the landscape, particularly in temperate and tropical regions. They therefore are recognized as being suitable for the reclamation of disturbed soils (Paschke, 1997). During the symbiosis with N_2-fixing actinomycetes of the genus *Frankia*, actinorhizal species can fix ecologically significant amounts of N_2 in woody root nodules (Paschke, 1997). Such species are generally woody species, with the exception of two sub-shrubs in the genus *Datisca.* Despite the relatively sporadic occurrence, the contribution of fixed N_2 to natural and managed landscapes by actinorhizal symbioses appears to be at least comparable to the relatively well-reported *Rhizobium*-legume contributions. The genera *Alnus,* for instance, was estimated to fix 12 to 200 kg of N ha^{-1} $year^{-1}$ and those of *Hippophae* associations 27 to 179 kg of N ha^{-1} $year^{-1}$ (Baker and Mullin, 1992). In various studies, the biological nitrogen fixation (BNF) potential was assessed as high for actinorhizal plants such as the genera *Casuarina* and *Alnus,* which have been studied most extensively (Brewbaker, 1989; Danso et al., 1992). Despite the species' widespread presence in the natural landscapes in Central Asia, studies on the actinorhizal symbiosis by *Elaeagnus angustifolia* L. from the *Elaeagnaceae* family are in their infancy and this species is still underrepresented in (actinorhizal) literature. Recent findings in Uzbekistan indicated the opportunities of actinorhizal *E. angustifolia* for use on degraded land despite the large variation in annually fixed N_2 amounts (Khamzina et al., 2009a), which increased from 24 in the first year to 514 kg ha^{-1} $year^{-1}$ in the third as quantified by the ^{15}N abundance technique in an open field trial.

Findings to date suggest that N_2-fixing *E. angustifolia* has the potential to be self-sufficient in N on the nutrient-exhausted irrigated croplands in Central Asia (Khamzina et al., 2009a). It is, however, unclear how to enhance juvenile growth of *E. angustifolia* or *R. pseudoacacia* at the lowest costs. This is of paramount importance for rapid income generation for land users, so they can profit from the sale of useful byproducts such as fuelwood, fruits and eventually timber (Lamers et al., 2008). Also the ecological service provision by these trees would be rendered at an earlier time. Nitrogen amendments would increase early growth and enhance tree stand establishment, but such a strategy is expensive and represses N_2 fixation (Fried and

[4] The term "actinorhizal" originates from the words roots "actino" indicating the *Frankia* actinomycete and "rhiza" indicating the plant roots hosting the symbiosis.

Broeshart, 1975) owing to the inhibitory effect of nitrate on both nodulation and nitrogenease activities (Streeter, 1988). Hence, this could be counterproductive. Some authors (e.g., Valladares et al., 2002) concluded that either high fertilization rates or inoculation can be recommended to enhance seedling growth of N_2-fixers, but that both methods are thus mutually exclusive. BNF by trees can be enhanced not only through the inoculation with selected symbiotic N_2-fixing bacteria, but also through appropriate management practices aiming at alleviating soil constraints such as deficiencies in limiting nutrients (Dommergues, 1995). Various edaphic factors affect N_2 fixation, such as excessive soil moisture, drought, soil acidity and deficiencies in calcium, molybdenum and boron, as well as phosphorus in acid soils or iron and zinc in alkaline soils (Mulongoy et al., 1992; Dommergues, 1995).

N_2-fixing annual and perennial plants have in general a high need for P (Marschner, 1986), and consequently BNF rates increase with P applications (Balasubramanian and Joshaline, 1996; Wheeler et al., 1996). On low P soils in West Africa, as little as 30 kg P ha^{-1} enhanced the growth of woody legumes (Sanginga, 2003). But information on the impact of P amendments and suitable P rates on symbiotic N_2 fixation by perennial species on the impoverished and saline soils of Central Asia is scarce. This is true for legumes (*Rhizobium*-legume symbiosis) and non-legumes (*Frankia*-nonlegume symbiosis). The salt-affected, degraded croplands in Central Asia suffer from water stress (be it in abundance or in short supply), or soil salinity. In saline environments, BNF is hampered mainly by osmotic moisture withdrawal from the nodules. The low soil P contents of these soils have an immediate impact on N_2 fixation, biomass accumulation and growth rates of the actinorhizal *E. angustifolia* or of the legume *R. pseudoacacia*.

Tools for plant growth analysis have been reported for annual and perennial vegetation (e.g., Evans, 1972; Hunt, 1982). In the case of deciduous trees such as *E. angustifolia* and *R. pseudoacacia*, total biomass is seasonal, and hence a growth analysis based on the relative growth rate (RGR) of the entire biomass carries the risk of underestimating the RGR of the truly perennating part of the tree material (Hunt, 1990). Consequently, in the case of perennial plants, RGR estimates need to be complemented with the unit production rate (UPR in mg g^{-1} day^{-1}), which indicates the rate of dry weight production of a tree (or tree stand) per unit of perennating weight. The UPR indicates the productive efficiency of the perennating biomass, which is known to decline with time (Dudney, 1974). Moreover, N productivity should be considered when analyzing and comparing growth performance (Lambers et al., 1998), e.g., between N_2–fixing species and non-fixing reference species.

Given the low soil P contents in the salt-affected, marginal croplands in Central Asia, an assessment was made of the impact of P amendments on the symbiotic performance of two N_2-fixing tree species and on the consequent production efficiency of the perennating and total biomass of these species compared to the performance of a non-fixing reference species. The objectives were: (1) to assess the effect of different P rates on the seasonal N_2 fixation by actinorhizal *E. angustifolia* and leguminous *R. pseudoacacia* grown on salt-affected agricultural land, (2) to quantify the contribution of N_2 fixation by two N_2-fixing tree species to the plant-soil system, (3) to evaluate the influence of P amendments on biomass production, relative growth rates and N productivity of these species compared to the non-fixing reference species *G. triacanthos*, and (4) to assess the suitability of the two N_2-fixing and the reference species for the afforestation of salt-affected croplands and for improving the landscape.

3.2 Materials and methods

3.2.1 Description on the study site

The research was conducted on 1.5 ha of degraded agricultural cropland in the Yangibazar district of the Khorezm region, located at 41°65′N, 60°62′E and 102 m altitude (Figure 2.1). The region has a continental climate with cold to very cold winters and very hot, dry summers. A weather station, installed in the northwestern part of the experimental site recorded at 30-min intervals: (i) air temperature, (ii) relative air humidity, (iii) energy balance between incoming and outgoing short- and long-wave infra red radiation, (iv) incoming shortwave radiation, (v) wind speed at 2-m height, and (vi) wind direction. A tipping-bucket rain gauge was used to monitor precipitation at each event. Annual precipitation during the study years 2006, 2007 and 2008 (Figure 3.1), which mostly occurs as rain and snowfall during the cold season (November-March) was lower than the long-term mean precipitation of 100 mm by 47, 51 and 64 %, respectively, which was much lower than the potential evapotranspiration of 1200-1500 mm (Conrad et al., 2007).

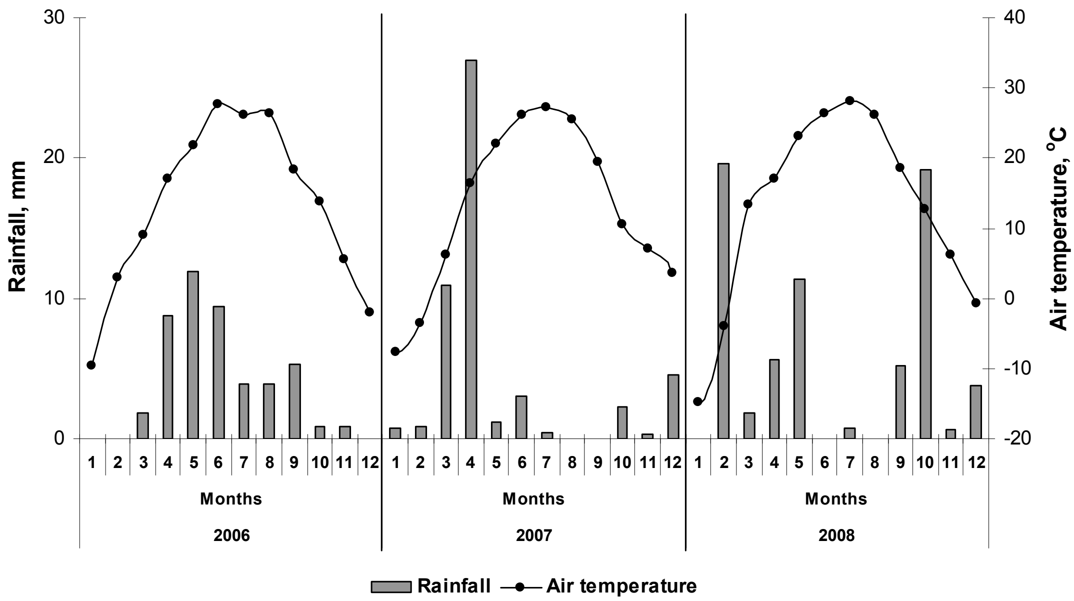

Figure 3.1: Monthly mean air temperature and precipitation at the Yangibazar experimental site in 2006, 2007 and 2008

The soil at the experimental site was an alluvial silt loam, traditionally used for irrigated meadows (Khamzina et al., 2006). The degree of soil salinity during 2006-2008 varied from moderate to strong (Table 3.1). Soil fertility was poor at the onset of the experiment as reflected in the low humus content (<1%), and low soil nitrogen (N), potassium (K) and phosphorus (P) concentrations, the latter being important for BNF (Table 3.1). Based on plant-available minerals in the plough layer (0-40 cm), a soil with an available P_2O_5 concentration ranging from 0-15 mg kg^{-1} and/or with exchangeable K_2O ranging from 0-100 mg kg^{-1} is classified as very low according to the local evaluation of soil fertility (Musaev, 2001). Based on this soil fertility classification, the soils in the experiment showed a low and very low fertility throughout the entire study period. The varying soil available P (P_2O_5) contents over the sampling periods may have been caused by the sampling procedure (see section 3.2.3 below). The decrease in exchangeable K_2O at the end of the study period, although statistically insignificant (Appendix 10.1), is likely due to tree uptake.

The groundwater table (GWT) at the onset of the 2006, 2007 and 2008 growing seasons averaged respectively 1.1, 1.4 and 1.3 m below the soil surface, with an average EC of 2.3 dS m^{-1}. Throughout the growing seasons of 2006 and 2007 the rooting depths of the trees were insufficient to tap the GWT. During these first two seasons tree development was sustained by drip irrigation of 80 mm per year.

Table 3.1: Electrical conductivity (EC, dS m^{-1}), humus content (%), concentration of total N (%), available P, exchangeable K (mg kg^{-1}), and Cl, Na, SO_4, Ca (cmol kg^{-1}) in 1-m soil layer at the onset and end of each growing season. Within a column, means followed by the same letter are not significantly different at *P*<0.05 according to the Tukey post-hoc test

Time	EC	Humus	N	P_2O_5	K_2O	Cl^-	Na^+	SO_4^{-2}	Ca^{+2}
May 2006	6.7 c	0.5 a	0.04 ab	7.0 b	109 a	0.5 a	1.9 b	2.9 b	2.05 b
Oct 2006	5.9 c	-	0.04 ab	11.9 ab	107 a	0.6 a	1.8 ab	2.6 b	1.61 b
May 2007	9.7 ab	0.6 a	0.04 ab	10.4 ab	96 ab	0.9 a	3.2 ab	4.0 ab	2.03 b
Oct 2007	10.6 a	0.6 a	0.05 a	7.7 b	104 ab	0.8 a	2.6 ab	3.4 b	2.08 b
May 2008	10.7 a	0.6 a	0.04 ab	12.6 a	102 ab	0.8 a	2.8 ab	3.6 b	2.19 ab
Oct 2008	7.5 bc	0.6 a	0.04 ab	9.7 ab	82 b	0.9 a	3.3 a	5.7 a	3.34 a
ANOVA, probability > F(=α)									
Sign.	<0.0001	0.196	0.015	0.052	0.004	0.083	0.029	<0.0001	<0.0001

Note: Borderline significant 0.05<P<0.1; significant: 0.001<P<0.05; highly significant: P<0.001

3.2.2 Experimental design

To assess the effects of P amendments on symbiotic performance and consequent tree growth, a 2-factorial field experiment was conducted over three consecutive years (2006-2008). The field trial included three P levels (i) “high-P” (the recommended 90 kg P ha^{-1}), (ii) “low-P” (50% of the recommended 90 kg P ha^{-1}), and (iii) the “nil” treatment (meaning no P applied, which served as the control, 0-P). Phosphorus was applied to three tree species, the actinorhizal Russian olive (*Elaeagnus angustifolia* L.), the leguminous black locust (*Robinia pseudoacacia* L.) and the non-fixer honey locust (*Gleditsia triacanthos L.*) as simple super phosphate (SSP; 16% P_2O_5) with the equivalent of 6.9 kg of pure P per 100 kg of SSP (P_2O_5 x 0.436 = P). Since tree fertilizer recommendations in Uzbekistan were developed for fruit species only (MAU, 1982), these guidelines for P amendments were adopted for the experiment. However, to encourage juvenile growth and minimize cost, the SSP was not broadcasted as recommended but instead locally applied, which is considered a more suitable application mode for fertilization of trees (Wray, 2001; Gilmann and Rosen, 2004). Based on the recommended 90 kg P ha^{-1} for a stand of 2860 fruit trees ha^{-1}, per planting hole 31.7 g of P was applied. Trees with the 0-P treatment did not receive P during the entire study period.

The trees were fertilized in March 2006 and April 2007. In 2006, P was placed in the planting hole of 50 cm depth prepared in advance to host a sapling. The fertilizer was mixed with part of the excavated soil and placed at the bottom of the hole before planting the tree sapling. In the second growing season 2007, a mixture of P and soil was applied into a 20-cm deep circle of 20-cm radius around each already established tree. Then this circle was backfilled with soil.

Each treatment was repeated four times. In March 2006, one-year-old saplings of all three species were transplanted from a tree nursery stock into the experimental plots 1 m apart in rows spaced 1.75 m (Figure 3.2), resulting in a stand density of 5,714 trees ha^{-1}. The trees at the beginning and end of each plot were considered border trees and therefore were excluded from in-depth measurements. The entire trial consisted of 48 experimental plots, each containing one row of 11 trees. However, from these 48 plots only 36 were considered further, since 12 plots were foreseen as “security” plots. This is recommended in particular for forestry experiments (Coe et al., 2003) in case, for instance, low survival rates in the trial plots would necessitate transplantation. Since none of the anticipated calamities occurred throughout the entire study period, these 12 plots were excluded from all measurements. During planting, 6 saplings of each species were analyzed separately for initial mean over-bark diameter, measured at 10 cm above the soil surface with a calliper, for height measurement with a measuring tape, and the for initial dry mass. After transplanting the saplings into the experimental plots, the trees were drip irrigated during 2006 and 2007 with 80 mm $year^{-1}$ (covering about 15% of evaporative demand). The emitters were installed about 10 cm from the tree basis. The amount of irrigation water was controlled through measuring valves. In 2008, irrigation was stopped as the trees started to rely on the groundwater; the GWT fluctuated during the growing seasons between 1.3 and 1.8 m.

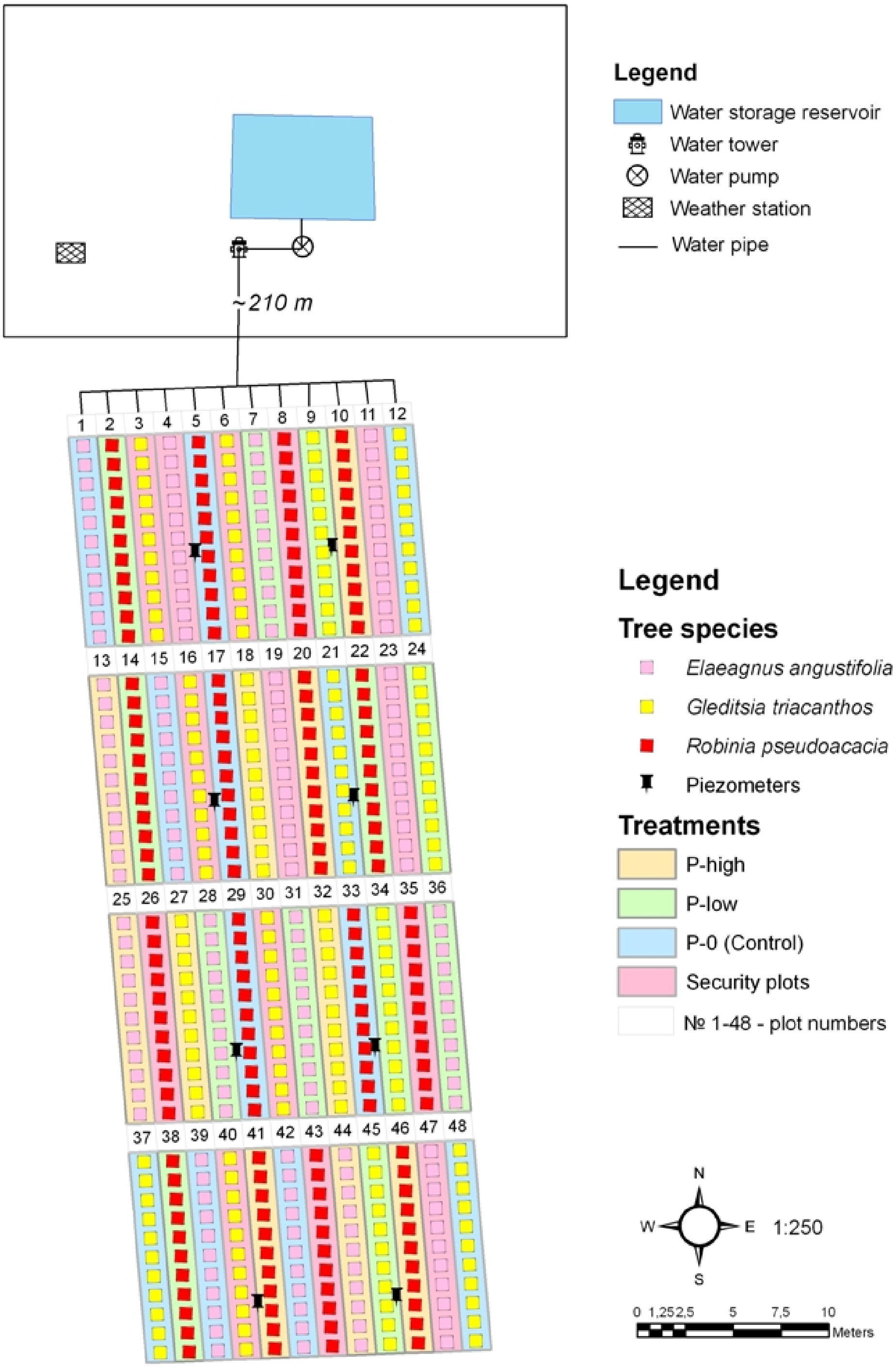

Figure 3.2: Experimental layout showing experimental plots, tree species, and treatments

3.2.3 Soil and groundwater sampling and analyses

Soil (in 0.2 m layers down to 1 m depth) was sampled at the end of each growing season from all 36 experimental plots and prepared for chemical analysis. The soil was sampled between the trees and thus outside the area where P was applied in 2006 and 2007. Statistical analyses showed that the differences within each sampling period were insignificant for the P treatments and tree species (Appendix 10.1). The findings were therefore averaged over the 1-m soil profile and over the 36 plots (Table 3.1) for each sampling period. The soil organic matter content was analyzed according to Tyurin (1975) and total N was determined using the Kjeldahl method. Available P (P_2O_2) was measured with a colorimeter in ammonium carbonate extract (Protasov, 1977). Exchangeable K (K_2O) was determined by flame photometry after extraction with 1% ammonium carbonate solution. In addition, twice a month during the growing season, soils from all 36 experimental plots were sampled in 0.2 m layers down to 1-m depth and analyzed for soil EC and moisture content. The EC of the soil water paste (1:1 ratio) was measured with a portable EC meter (Shirokova et al., 2000) and converted into EC_e using the relationship [$EC_e=EC_{1:1}\cdot 3.6$] as proposed for sandy loam soils in Khorezm. Soil moisture was measured gravimetrically by oven drying at 103 °C ± 2 °C to constant weight in a forced air convection oven. Samples were weighed before and after drying with a digital balance to the nearest mg.

The GWT was permanently monitored through eight observation wells installed at 2.2 m depth. The GW level was registered every ten days during when the GW samples were collected. These were analyzed for EC with a portable EC meter (Shirokova et al., 2000) and converted to into EC_e as previously described.

3.2.4 Determination of dry matter and biomass accumulation

At the end of the three growing seasons, two trees in each of the 36 plots were cut at ground level and separated into leaves, twigs, stems and fruits. Following the harvest of the aboveground fractions, the tree roots were completely excavated and separated into coarse (Ø>3 mm) and fine (Ø<3 mm) roots and nodules. The roots were washed and freed from soil and weighed with a digital scale to the nearest mg. Coarse root length was measured with a measuring tape while the fine root length was determined with the modified Newman line-intersect method (Tennant, 1975). All above- and belowground fractions were placed in paper bags and oven dried at 103 °C for 72 hours to constant weight. The initial weight of the trees (mean of 6 trees per species) measured in the beginning of 2006 was subtracted from the total biomass of the trees at the end of the same year. Thereafter, the dry matter (DM) production of all tree species was converted from g $tree^{-1}$ to kg ha^{-1} using the stand density of 5,714 trees ha^{-1}. The relative relationship of below- and aboveground DM was expressed as root to shoot ratio (RSR).

3.2.5 Height and diameter

The initial height and diameter of the planted saplings were determined at the onset (one week after planting) of the first growing season 2006. Thereafter, tree height and diameter were measured fortnightly throughout the three growing seasons, excluding the four winter months (November-March). The height was measured from the soil level to the highest vegetative point with a telescope measuring stick to the nearest cm. Concurrently, the over-bark stem diameter was measured to the nearest mm at 10 cm above the stem base ($diam_{10}$) using a digital tree caliper. The measurement points for diameter had been permanently marked on the tree stems at the first measuring date and were used throughout the entire study period of three years.

3.2.6 Estimating the symbiotic fixation of the N_2-fixing tree species

To estimate the end-of-season N_2 fixation by *E. angustifolia* and *R. pseudoacacia*, leaves, twigs, stems, coarse and fine roots and nodules of the harvested trees of both N_2-fixers and the reference species *G. triacanthos* were sampled separately for the three P application levels in four replications. Due to the small size of the tree saplings in 2006, the fractions were separated into leaves, twigs and fine roots only. The sampled tree parts were transported to the laboratory in paper bags and oven dried at 60 °C for 72 hours to constant weight. The bark was not separated from the stem. Thereafter, the dried samples were finely ground in a mill to pass through a 2-mm mesh screen and analyzed for N and ^{15}N natural abundance with an ANCA mass spectrometer (SL/20-20, SerCon, UK).

The N_2-fixation of *E. angustifolia* and *R. pseudoacacia* was quantified with the ^{15}N natural abundance method according to Shearer and Kohl (1986):

$$\%Ndfa = \left(\frac{(\delta^{15}N_{ref} - \delta^{15}N_{fixer})}{(\delta^{15}N_{ref} - B)} \right) 100 \tag{3.1}$$

where %Ndfa is the proportion of N derived from atmospheric N_2, $\delta^{15}N_{ref}$ is the natural abundance of the non-fixing reference species *G. triacanthos*, and $\delta^{15}N_{fixer}$ is the $\delta^{15}N$ of the N_2-fixing *E. angustifolia* and *R. pseudoacacia*. The parameter *B* is the ^{15}N value of the same N_2-fixing species grown in an N-free culture. Since the *B* values were unavailable for the examined species grown in Central Asia, they needed to be derived from secondary sources. For each year, %Ndfa of *E. angustifolia* was calculated using a weighted mean of $\delta^{15}N$ based on whole-plant *B* values, which ranged from -1.41 to -2.0 as previously reported for actinorhizal species (Domenach et al., 1989; Tjepkema et al., 2000). As was done by Khamzina et al. (2009a) for foliar %Ndfa by *E. angustifolia*, the minimal field-observed foliar $\delta^{15}N$ of -2.67 as reported by Peoples et al. (2002) was used as the *B* value. For the estimation of foliar as well as whole-tree %Ndfa of leguminous *R. pseudoacacia*, the *B* value of -1.35 and -2, respectively, were used based on the value reported specifically for legume species by Shear and Kohl (1986). The annual rates of N_2 fixation (expressed in kg ha^{-1} $year^{-1}$) by *E. angustifolia* and *R. pseudoacacia* were calculated using the estimated %Ndfa, the annually accumulated N content, and a tree density of 5,714 trees ha^{-1} according to:

$$Ndfa\ (kg\ ha^{-1}) = (\%Ndfa \cdot total\ N_{fixer}) / 100 \tag{3.2}$$

For calculating N_2 fixation for the whole tree, a weighted mean (rather than an arithmetic mean of individual parts) was used based on the following equation (3.3):

$$WM\ Ndfa\ (kg\ ha^{-1}) = \frac{\%Ndfa_L \cdot N_L + \%Ndfa_T \cdot N_T + \%Ndfa_S \cdot N_S + \%Ndfa_{CR} \cdot N_{CR} + \%Ndfa_{FR} \cdot N_{FR} + Ndfa_N \cdot N_N}{(N_L + N_T + N_S + N_{CR} + N_{FN} + N_N)} \tag{3.3}$$

where %Ndfa is the proportion of N derived from atmospheric N_2, N is the total N yield, whilst L, T, S, CR, FR, and N stand for leaves, twigs, stem, coarse roots, fine roots and nodules, respectively.

3.2.7 Growth rates

Unit production rate (UPR) was calculated according to Hunt (1982; 1990) as:

$$\Pi = (W_2\text{-}W_1)/(t_2\text{-}t_1) \cdot (\ln W_{P2}\text{-}\ln W_{P1})/(W_{P2}\text{-}W_{P1}) \qquad (3.4)$$

where W_1 and W_2 are the initial and subsequent dry weight of trees, t_1 and t_2 are the initial and subsequent time of harvest, and W_{P1} and W_{P2} are the rates of dry weight production of trees expressed per unit of perennating structure.

Similarly, the relative growth rate (RGR) was estimated over the variables height and diameter while accounting for the variable time as:

$$RGR_H \text{ (in mm mm}^{-1}\text{ d}^{-1}\text{): Relative height growth rate } (\ln H_2 - \ln H_1)/(t_2 - t_1) \qquad (3.5)$$

$$RGR_D \text{ (in mm mm}^{-1}\text{ d}^{-1}\text{): Relative diameter growth rate } (\ln D_2 - \ln D_1)/(t_2 - t_1) \qquad (3.6)$$

where H_1 and H_2 are the initial and subsequent height, and D1 and D2 the initial and subsequent diameter ($diam_{10}$) at the time of harvest at t_1 and t_2.

The determination of nitrogen productivity (NP) followed the relation suggested by Lambers et at. (1998):

$$NP = RGR/PNC \qquad (3.7)$$

where RGR (mg g^{-1} day^{-1}) is the relative growth rate and PNC is the plant N concentration, i.e., total plant N per total plant mass.

3.2.8 Statistical analyses

Descriptive statistics including the (least square) means and standard deviation of measured tree variables were computed each year for eight individual trees per treatment. All variables were tested for normal distribution visually and by using the Kolmogorov-Smirnov test and normalized by square-root transformations when necessary. Mean separation tests through analyses of variance (ANOVA) were conducted using transformed data, but data presented are the untransformed means as suggested by Steel and Torrie (1980). In case of occasional missing values, least square means are reported. Similarly, owing to occasional missing values, the Tukey post-hoc test was used to assign year, species and treatments to statistically different groups. The significance level was set at $P<0.1$ unless mentioned otherwise. All statistical analyses were performed with SPSS 17.0. Under the assumption of equal variances, a student's *t*-test was carried out to examine differences between foliar and whole-tree %Ndfa and total fixed N_2 values.

3.3 Results

3.3.1 Impact of P amendments on nitrogen fixation of N_2-fixing tree species

The end-of-season foliar and whole tree ^{15}N natural abundance values of both N_2-fixing *E. angustifolia* and *R. pseudoacacia* were negative and significantly different from the non-fixing *G. triacanthos* when averaged over the years and treatments (Table 3.2). Furthermore, the differences in foliar and whole-tree $\delta^{15}N$ between the two N_2-fixing species were significant, with *E. angustifolia* having the highest negative values both for foliar and whole-tree $\delta^{15}N$. When averaged over all species and P treatments, the $\delta^{15}N$ in 2008 was significantly higher than in 2006 and 2007. The differences between the much lower values in the earlier years were insignificant. The $\delta^{15}N$ values were not affected by P amendments.

Due to a significant species*year interaction (Table 3.2), findings were further disaggregated (Table 3.3). In 2006, the differences between $\delta^{15}N$ among tree fractions of the two N_2-fixing species were insignificant, but leaves of the reference tree species *G. triacanthos* showed significantly higher ^{15}N abundance values than all other tree fractions examined. From all tree fractions analyzed in 2007, the twigs, stems and nodules of *E. angustifolia* had the lowest ^{15}N abundance. In 2008, the nodules of *E.*

angustifolia were most depleted in ^{15}N, followed by fine roots. In 2007, the stems of *R. pseudoacacia* had the lowest $^{15}\delta N$ whereas the leaves of this species had the highest value. Similar to *E. angustifolia*, the ^{15}N depletion was the highest in the nodules of *R. pseudoacacia* in 2008. Fine roots of the reference species *G. triacanthos* showed the highest ^{15}N abundance value in 2007, whereas in 2008, the $\delta^{15}N$ among fractions of this species did not vary significantly (Table 3.3).

Table 3.2: Least square means of the end-of-season foliar and whole-tree $\delta^{15}N$ values as affected by species, year and phosphorus application. Within a column segments delineated by each factor (species, year, treatment), the means followed by the same letter (a, b, c) are not significantly different at $P<0.1$ according to the Tukey post-hoc test

Factor	Variable	Foliar mean	Whole-tree mean
			$\Delta^{15}N$
Species	*Elaeagnus angustifolia*	-1.77 c	-1.81 c
	Robinia pseudoacacia	-0.87 b	-1.48 b
	Gleditsia triacanthos	2.21 a	1.89 a
Year	2006	-0.11 a	-0.31 a
	2007	-0.08 a	-0.32 a
	2008	-0.63 b	-0.95 b
P treatment	0-P	-0.17 a	-0.58 a
	Low-P	-0.34 a	-0.64 a
	High-P	-0.32 a	-0.53 a
		ANOVA, probability > F(=α)	
Species		< 0.0001	< 0.0001
Year		< 0.0001	< 0.0001
P treatment		0.147	0.193
Species*year		< 0.0001	< 0.0001
Year*P treatment		0.510	0.641
Species*P treatment		0.457	0.543

Note: Borderline significant 0.05<P<0.1; significant: 0.001<P<0.05; highly significant: P<0.001

The overall results showed that the non-N-fixer *G. triacanthos* is a suitable reference tree species given its constant positive ^{15}N values, thus indicating no N_2 fixation both on the basis of whole trees (Table 3.2) and fractions (Table 3.3).

3.3.2 Impact of P amendments on nitrogen concentrations

When combined over the years, the weighted mean of N concentrations accumulated over all fractions varied considerably among tree species, with the highest values in N_2-fixing *E. angustifolia* and *R. pseudoacacia* and the lowest in the non-fixing *G.*

triacanthos (Table 3.4). When differentiated by fractions, the two N_2-fixers had statistically either similar N concentrations in some fractions (e.g., in leaves and fine roots), or *R. pseudoacacia* had lower (e.g., in twigs) or higher N (e.g., in stems, coarse roots) concentrations than *E. angustifolia*.

Table 3.3: $\delta^{15}N$ in tree fractions of *E. angustifolia*, *R. pseudoacacia* and *G. triacanthos* at the end of the 2006, 2007 and 2008 growing seasons. Within a row, means followed by the same letter are not significantly different at *P*<0.1 according to the Tukey post-hoc test

Year	Species	Tree fractions					
		Leaves	Twigs	Stems	Fine roots	Coarse roots	Nodules
2006	*E. angustifolia*	-1.41 a	-1.31 a	-	-1.16 a	-	-
	R. pseudoacacia	-0.86a	-0.93 a	-	-1.03 a	-	-
	G. triacanthos	2.54 a	1.13 b	-	0.89 b	-	-
2007	*E. angustifolia*	-2.15 ab	-2.33 a	-2.50 a	-1.68 b	-1.75 b	-2.33 a
	R. pseudoacacia	-0.73 d	-1.52 bc	-2.33 a	-1.27 ab	-1.98 cd	-1.83 cd
	G. triacanthos	2.76 ab	1.94 c	2.12 bc	3.05 a	1.79 c	na
2008	*E. angustifolia*	-1.73 ab	-1.60 ab	-1.72 ab	-2.06 b	-1.55 c	-2.81 a
	R. pseudoacacia	-1.03 c	-1.94 b	-1.61 bc	-1.85 bc	-1.43 cd	-2.52 a
	G. triacanthos	1.32 a	1.78 a	1.80 a	2.00 a	1.41 a	na

	ANOVA, probability > F(=α)		
Factor	2006	2007	2008
Species	< 0.0001	< 0.0001	< 0.0001
Fractions	< 0.0001	< 0.0001	< 0.0001
Species*fractions	< 0.0001	< 0.0001	< 0.0001

Note: Borderline significant 0.05<P<0.1; significant: 0.001<P<0.05; highly significant: P<0.001

Both on the basis of the whole tree and the tree fractions, the highest N concentrations were found with the high-P treatment (Table 3.4). Nitrogen concentrations in the control (0-P) trees and their fractions were always significantly lower than with high-P except for the concentrations in the stem. The concentrations with low-P did not differ from 0-P and high-P. On a whole-tree basis, the differences in N concentrations were insignificant between years, whereas the N concentrations in fractions varied differentially between the years. Hence the overall findings indicated that due to N_2 fixation, N concentrations with high-P significantly increased in all fractions but particularly in leaves and fine roots (both 18%) compared to P-0.

Table 3.4: Least square means of nitrogen concentrations (%) in tree fractions as affected by species, level of phosphorus application and years. Within a column segment delineated by each factor (species, treatment, year), means followed by the same letter are not significantly different at $P<0.1$ according to the Tukey post-hoc test

Factor	Variable	Leaves	Twigs	Stems	Coarse roots	Fine roots	Weighted mean
		N concentration, %					
Species	*E. angustifolia*	2.76 a	0.93 a	0.55 b	1.40 b	1.99 a	1.61 a
	G. triacanthos	1.38 b	0.56 c	0.50 c	0.67 c	0.73 b	0.80 b
	R. pseudoacacia	2.64 a	0.87 b	0.63 a	1.75 a	1.99 a	1.63 a
	P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
		N concentration, %					
P treatment	0-P	2.06 b	0.76 b	0.54 b	1.21 b	1.45 b	1.25 b
	Low-P	2.29 a	0.77 b	0.57 a	1.25 b	1.54 b	1.34 ab
	High-P	2.43 a	0.82 a	0.56 b	1.36 a	1.72 a	1.44 a
	P value	0.01	0.02	0.06	0.007	0.013	0.12
		N concentration, %					
Year	2006	2.14 b	0.86 a	na	na	0.98 b	1.32 a
	2007	2.49 a	0.68 c	0.55 a	1.31 a	1.83 a	1.37 a
	2008	2.15 b	0.81 b	0.57 a	1.24 b	1.90 a	1.33 a
	P value	<0.0001	<0.0001	0.17	0.09	<0.0001	0.85

Note: Borderline significant 0.05<P<0.1; significant: 0.001<P<0.05; highly significant: P<0.001. na – not available

The analysis also showed that compared to 0-P, high-P significantly increased leaf N content of *E. angustifolia*, while the effect of high-P was not confirmed for *R. pseudoacacia* (Table 3.5).

3.3.3 Impact of P amendments on N_2 fixation

When averaged over all years and treatments, Ndfa (%) as well as the amount of N_2 fixed (kg ha^{-1}) by *E. angustifolia* was significantly higher than by *R. pseudoacacia*. At all measuring times, the Ndfa (kg ha^{-1}) with high-P was significantly higher than with low-P and 0-P when combined over all years and species. The exception was foliar %Ndfa, which significantly increased with high-P compared to 0-P. When compared on a whole-tree basis (weighted means) the Ndfa (kg ha^{-1}) was significantly different for all P treatments, with the highest Ndfa with high-P and the lowest with 0-P. The foliar %Ndfa increased over years and peaked in 2007 while the whole-tree weighted mean of %Ndfa increased over years and peaked in 2008 (Table 3.6). The Ndfa (kg ha^{-1}) based on the foliar as well as the whole-tree weighted means increased in the order of: 2006<2007<2008 (Table 3.6).

Over three years, high-P significantly increased the foliar as well as whole-tree %Ndfa of *E. angustifolia* when compared to 0-P. In the case of *R. pseudoacacia*, the foliar %Ndfa was not affected by P additions, whereas the whole-tree N_2 fixation significantly increased with low-P and high-P (Figure 3.3).

Table 3.5: Least square means of tissue nitrogen concentration (%) as affected by years, species and phosphorus application interactions. Within a column, means followed by the same letter are not significantly different at P<0.1 according to the Tukey post-hoc test

Interaction/ Variable		Leaves	Twigs	Stem	Coarse roots	Fine roots	Weighted mean
Year*Species		N concentration, %					
2006	*Elaeagnus angustifolia*	3.05 a	0.97 a	na	na	1.33 a	1.79 a
	Gleditsia triacanthos	1.27 c	0.67 b	na	na	0.44 b	0.80 c
	Robinia pseudoacacia	2.09 b	0.94 a	na	na	1.18 a	1.41 b
	P value	<0.0001	<0.0001	-	-	<0.0001	<0.0001
2007	*E. angustifolia*	2.97 a	0.86 a	0.62 a	1.46 b	2.20 a	1.62 a
	G. triacanthos	1.50 b	0.40 b	0.40 b	0.62 c	0.91 b	0.77 b
	R. pseudoacacia	3.00 a	0.78 a	0.63 a	1.84 a	2.39 a	1.73 a
	P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
2008	*E. angustifolia*	2.25 a	0.95 a	0.48 b	1.35 b	2.46 a	1.50 a
	G. triacanthos	1.37 b	0.60 b	0.60 a	0.72 c	0.83 b	0.82 b
	R. pseudoacacia	2.82 a	0.89 a	0.63 a	1.65 a	2.40 a	1.68 a
	P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Overall	***P* value**	**<0.0001**	**0.21**	**<0.0001**	**0.02**	**<0.0001**	**0.11**
Year*P treatment							
2006	0-P	1.89 b	0.86 a	na	na	1.03 a	1.26 a
	Low-P	2.22 b	0.85 a	na	na	0.94 a	1.34 a
	High-P	2.31 a	0.87 a	na	na	0.98 a	1.39 a
	P value	0.04	0.98	-	-	0.89	0.78
2007	0-P	2.30 b	0.66 a	0.53 a	1.31 a	1.70 b	1.30 a
	Low-P	2.48 b	0.68 a	0.56 a	1.28 a	1.72 b	1.34 a
	High-P	2.68 a	0.70 a	0.56 a	1.33 a	2.08 a	1.47 a
	P value	0.08	0.91	0.83	0.97	0.06	0.58
2008	0-P	2.00 a	0.75 b	0.54 a	1.11 b	1.63 b	1.21 a
	Low-P	2.15 a	0.79 b	0.59 a	1.22 b	1.98 b	1.35 a
	High-P	2.29 a	0.90 a	0.58 a	1.39 a	2.09 a	1.45 a
	P value	0.55	0.10	0.41	0.001	0.01	0.22
Overall	***P* value**	**0.99**	**0.75**	**0.94**	**0.64**	**0.65**	**0.97**
Species*P treatment							
E. angustifolia	0-P	2.49 b	0.91 a	0.55 a	1.35 a	1.89 a	1.52 a
	Low-P	2.70 b	0.92 a	0.56 a	1.38 a	2.09 a	1.62 a
	High-P	3.07 a	0.95 a	0.53 a	1.48 a	2.00 a	1.70 a
	P value	0.01	0.77	0.66	0.14	0.68	0.54
G. triacanthos	0-P	1.26 a	0.53 a	0.46 a	0.57 b	0.60 b	0.71 a
	Low-P	1.40 a	0.57 a	0.52 a	0.75 a	0.68 ab	0.81 a
	High-P	1.47 a	0.56 a	0.52 a	0.70 ab	0.91 a	0.87 a
	P value	0.16	0.79	0.51	0.09	0.03	0.12
R.pseudoacacia	0-P	2.43 a	0.82 b	0.60 a	1.72 ab	1.87 b	1.54 a
	Low-P	2.75 a	0.83 b	0.64 a	1.61 b	1.86 b	1.60 a
	High-P	2.74 a	0.96 a	0.65 a	1.91 a	2.24 a	1.76 a
	P value	0.33	0.05	0.18	0.07	0.07	0.40
Overall	***P* value**	**0.49**	**0.44**	**0.69**	**0.11**	**0.71**	**0.98**

na – not available

Table 3.6: Least square means of nitrogen fixation (Ndfa %, kg ha^{-1}) as affected by species, level of phosphorus application and years. Within a column segment delineated by each factor (species, treatment, year) means followed by the same letter are not significantly different at P<0.1 according to the Tukey post-hoc test

Factor	Variable	Foliar	Whole-tree	Foliar	Whole-tree
		Ndfa (%)		Ndfa (kg ha^{-1})	
Species	*E. angustifolia*	84 a	87 a	209 a	285 a
	R. pseudoacacia	59 b	82 b	44 b	57 b
P treatment	0-P	69 b	77 b	89 b	113 c
	Low-P	71 ab	84 a	120 b	169 b
	High-P	75 a	85 a	169 a	230 a
Year	2006	57 c	68 c	17 c	26 c
	2007	82 a	87 b	77 b	121 b
	2008	76 b	92 a	284 a	365 a
		ANOVA, probability > F(=α)			
Year		< 0.0001	< 0.0001	< 0.0001	< 0.0001
Species		< 0.0001	< 0.0001	< 0.0001	< 0.0001
P treatment		0.074	< 0.0001	< 0.0001	< 0.0001
Year*species		< 0.0001	< 0.0001	< 0.0001	< 0.0001
Year*P treatment		0.698	0.939	0.021	< 0.0001
Species*P treatment		0.183	0.750	0.089	< 0.0001

Note: Borderline significant 0.05<P<0.1; significant: 0.001<P<0.05; highly significant: P<0.001

Due to a significant year*treatment interaction in the amount of N_2 fixation (kg ha^{-1}) based both on foliar and weighted whole-tree means (Table 3.6), these parameters were disaggregated and analyzed according to species in each year and treatment (Table 3.7).

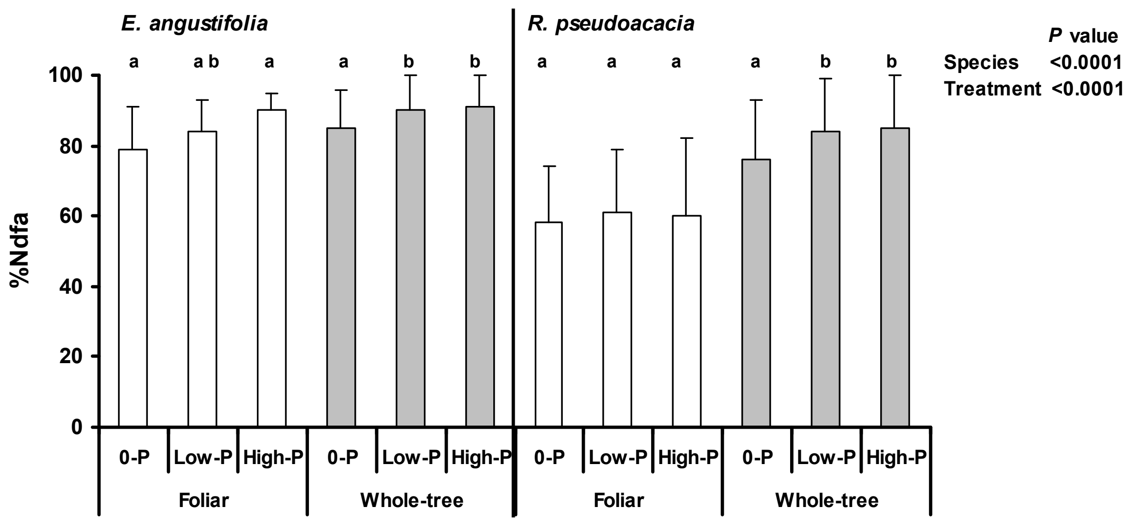

Figure 3.3: Nitrogen fixation (%Ndfa) of *E. angustifolia* and *R. pseudoacacia* with three levels of P application (0-P, low-P and high-P) based on foliar and whole-tree ^{15}N natural abundance referenced against *G. triacanthos* over three years. Vertical bars indicate standard deviations of the means

Table 3.7: Least square means in 2006, 2007 and 2008 of foliar and whole-tree nitrogen fixation (kg ha^{-1}) by *E. angustifolia* and *R. pseudoacacia* according to three levels of P application. Within a column delineated by each factor, means followed by the same letter are not significantly different at *P*<0.1. The overall means of Ndfa within a column and each year followed by different capital letter are significantly different at *P*<0.1 according to the Tukey post-hoc test

Species		2006	
	P treatment	Foliar	Whole-tree
		Ndfa (kg ha^{-1})	
Elaeagnus angustifolia	0-P	14 b	25 b
	Low-P	34 b	53 a
	High-P	43 a	64 a
Overall means		**30 C**	**48 C**
		2007	
Elaeagnus angustifolia	0-P	79 b	103 b
	Low-P	119 ab	195 a
	High-P	168 a	269 a
Overall means		**122 B**	**190 B**
		2008	
Elaeagnus angustifolia	0-P	370 b	445 b
	Low-P	433 b	599 b
	High-P	618 a	807 a
Overall means		**474 A**	**617 A**
		2006	
Robinia pseudoacacia	0-P	1 b	2 b
	Low-P	2 b	4 b
	High-P	3 a	9 a
Overall means		**2 C**	**5 C**
		2007	
Robinia pseudoacacia	0-P	24 b	30 c
	Low-P	32 b	49 b
	High-P	42 a	78 a
Overall means		**33 B**	**53 B**
		2008	
Robinia pseudoacacia	0-P	47 b	72 b
	Low-P	97 a	111 ab
	High-P	142 a	155 a
Overall means		**95 A**	**113 A**
		ANOVA, probability > F(=α)	
Year		< 0.0001	< 0.0001
Species		< 0.0001	< 0.0001
P treatment		< 0.0001	< 0.0001
Year*species		< 0.0001	< 0.0001
Year*P treatment		0.021	< 0.0001
Species*P treatment		0.089	0.040

Note: Borderline significant 0.05<P<0.1; significant: 0.001<P<0.05; highly significant P<0.001

The foliar Ndfa (kg ha^{-1}) by *E. angustifolia* with high-P in 2006 was significantly higher than with low-P and 0-P. In 2007 and 2008, this was essentially the same. On the other hand, the whole-tree Ndfa (kg ha^{-1}) by *E. angustifolia* estimated with high-P and low-P in 2006 and 2007 was significantly higher than with 0-P. With time, the effect of the higher P rate became increasingly evident, and in 2008, the Ndfa (kg ha^{-1}) was significantly higher with high-P, exhibiting a N_2 fixation of as high as 807 kg ha^{-1}. This amounts to an average of about 270 kg ha^{-1} $year^{-1}$. The overall means of Ndfa showed a significant increase in N_2 fixation over the years both in leaves and the whole tree. The whole-tree Ndfa in 2008 was ca. 23% higher than that of the foliar Ndfa.

In 2006 and 2007, *R. pseudoacacia* had significantly higher amounts of N_2 fixed in the leaves with high-P, whilst the difference in N_2 fixation between low-P and 0-P was insignificant. The same tendency was observed in 2006 when estimated on a whole-tree basis. In 2008, the foliar Ndfa with high-P and low-P was significantly higher compared to 0-P. The whole-tree Ndfa differed significantly among the P treatments, showing the highest values with high-P in 2007, whereas after three years there was a significant difference only between high-P (155 kg ha^{-1}: average 52 kg ha^{-1} $year^{-1}$) and 0-P, the latter being the lowest (72 kg ha^{-1}: average 24 kg ha^{-1} $year^{-1}$). The difference between low-P and 0-P was insignificant. The foliar as well as the whole-tree N_2 fixation by *R. pseudoacacia* increased significantly over years and peaked in 2008 (Table 3.7). The whole-tree Ndfa in 2008 was ca. 19% higher than that of the foliar Ndfa.

The end-of-season Ndfa (%, kg ha^{-1}) of *E. angustifolia,* estimated with the student's *t*-test, revealed a significant difference between foliar and whole-tree N_2 fixation over three years when referenced against *G. triacanthos* ($P(T<=t)$=0.014 and $P(T<=t)$=0.018, respectively), whereas for *R. pseudoacacia* the difference for %Ndfa was insignificant ($P(T<=t)$=1.137). However, Ndfa (kg ha^{-1}) by the leguminous species estimated on the whole-tree basis was significantly higher than that estimated based on leaves ($P(T<=t)$=0.012).

3.3.4 Impact of P application on biomass production, relative growth rates and N productivity

Biomass production

The biomass production of the three tree species differed significantly when values were combined over three years and three P amendments and compared on the basis of tree fractions and whole trees (Table 3.8).

Table 3.8: Least square means of dry matter production (t ha^{-1}) separated into fractions, above- and belowground and total biomass as affected by species, phosphorus application and years. Within a column and each factor, means followed by the same letter are not significantly different at $P<0.1$ according to the Tukey post-hoc test

Factor	Variable	Leaves	Twigs	Stem	Coarse roots	Fine roots	Stump	Above ground	Below ground	Total biomass
		Dry matter production, t ha^{-1}								
Species	*Elaeagnus angustifolia*	7.28 a	17.24 a	20.67 a	3.55 a	0.33 a	5.84 a	47.51 a	3.87 a	51.34 a
	Gleditsia triacanthos	0.14 c	0.34 c	0.08 c	0.16 c	0.05 c	0.55 c	0.92 c	0.20 c	1.13 c
	Robinia pseudoacacia	1.81 b	2.82 b	2.24 b	1.46 b	0.16 b	1.82 b	8.09 b	1.62 b	9.71 b
	P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
P treatment	0-P	2.60 b	6.55 b	6.59 a	2.02 a	0.19 a	2.79 b	17.26 b	2.21 a	19.47 b
	Low-P	2.96 b	5.93 b	6.89 a	1.41 a	0.18 a	2.27 b	17.41 b	1.59 b	18.99 b
	High-P	3.66 a	7.72 a	8.39 a	1.73 a	0.17 a	3.13 a	21.84 a	1.89 ab	23.74 a
	P value	0.009	0.004	0.73	0.16	0.68	0.005	0.0011	0.08	0.020
Year	2006	0.38 c	0.61 c	0.20 c	0.40 c	0.17 b	na	1.19 c	0.57 c	1.76 c
	2007	2.58 b	5.26 b	3.36 b	1.27 b	0.23 a	1.40 b	12.63 b	1.49 b	14.12 b
	2008	6.26 a	14.21 a	18.70 a	3.50 a	0.14 b	3.97 a	42.69 a	3.63 a	46.33 a
	P value	<0.0001	<0.0001	<0.0001	<0.0001	0.016	<0.0001	<0.0001	<0.0001	<0.0001

Note: Borderline significant 0.05<P<0.1; significant: 0.001<P<0.05; highly significant: P<0.001. na – not available

The highest biomass accumulation was observed in the N_2-fixer *E. angustifolia*, which was 81% higher than that of the other N_2-fixer *R. pseudoacacia* and 97% higher compared than that of the non-fixer *G. triacanthos* (Table 3.8). The bulk of the total DM was accumulated in the aboveground fractions and amounted to 93% of the total biomass in *E. angustifolia*, 81% in *G. triacanthos* and 83% in *R. pseudoacacia*. When combined over the three species and three years, the high-P treatment had a significantly higher total DM production compared to 0-P and low-P, whereas the differences between 0-P and low-P were statistically insignificant (Table 3.8).

There was a clear positive effect of P rates on leaves (P=0.009), twigs (P=0.004), stump (P=0.005) and aboveground biomass (P=0.0011). Except for the fine roots, biomass production of all tree fractions increased over time (Table 3.8).

Below- and above-ground biomass partitioning

The root/shoot ratio (RSR) of *E. angustifolia* declined with time (Table 3.9). In 2006, this species produced relatively more belowground DM, as suggested by the highest RSR of 0.43. In the following two years, the growth of *E. angustifolia* was shifted towards relatively more aboveground DM with a lower RSR of 0.12 in 2007 and 0.08 in 2008. A similar tendency was observed for *R. pseudoacacia* and *G. triacanthos* during the establishment year, although the RSR of these two species was significantly higher than that of *E. angustifolia*. In 2007, the RSR of *G. triacanthos* and *R. pseudoacacia* declined, indicating once more a relative favoring of aboveground DM. In 2008, the RSR values were similar to those in 2007 for *G. triacanthos*, whereas for *R. pseudoacacia* there was a small increase of 15%. Phosphorus amendments did not affect the RSR in the species, which was consistent over the years.

Conventional growth assessment parameters

The growth assessment based on the unit production rate (UPR) and the conventional parameters favored by foresters such as percentage increase in height (RGR_H) and diameter (RGR_D) showed significant differences among years, species and P amendments (Table 3.10).

Table 3.9: Root/shoot ratios of three tree species according to the study years and tree species. Within a column and each year, means followed by the same letter are not significantly different at *P*<0.05 according to the Tukey post-hoc test

Year	Species	Root/Shoot ratio
2006	*E. angustifolia*	0.43 b
	G. triacanthos	2.77 a
	R. pseudoacacia	2.00 a
2007	*E. angustifolia*	0.12 a
	G. triacanthos	0.15 ab
	R. pseudoacacia	0.17 a
2008	*E. angustifolia*	0.08 a
	G. triacanthos	0.15 ab
	R. pseudoacacia	0.20 b
	ANOVA, probability > F(=α)	
Year		< 0.0001
Species		0.001
P treatment		0.875
Year*species		< 0.0001
Year*P treatment		0.976
Species*P treatment		0.868

Note: Borderline significant 0.05<P<0.1; significant: 0.001<P<0.05; highly significant: P<0.001

Phosphorus applications did not significantly affect the UPR of *E. angustifolia* in all three years. However, in 2006, RGR_H and RGR_D with high-P were significantly higher than with 0-P. In 2007, the highest RGR_H was with high-P as well as with low-P, but in 2008 the high-P treatments exhibited superior RGR_H. The UPR for the non-fixer *G. triacanthos* decreased over the years, showing a great difference between high-P and 0-P in 2006. The RGR_H and RGR_D values were significantly higher with high-P in 2006, 2007 and 2008, except for RGR_H in 2008. In 2006, *R. pseudoacacia* showed the highest UPR with high-P and the lowest with 0-P. The same was observed for RGR_H and RGR_D. In 2007, though the lowest UPR was observed with high-P, the low-P and 0-P values were statistically similar. In the following year, the high-P again showed the highest and 0-P the lowest values. The relative growth in height and diameter of this species was not affected by P amendments in 2007.

Table 3.10: Species means of unit production rate (UPR) and relative growth rate in height (RGR_H) and diameter (RGR_D) according to P treatments in 2006, 2007 and 2008. Within a column and each tree species, values followed by the same letter are not significantly different at *P*<0.05 according to the Tukey post-hoc test

Species	P treatment	UPR ($mg\ g^{-1}\ d^{-1}$)	RGR_H ($mm\ mm^{-1}\ d^{-1}$)	RGR_D ($mm\ mm^{-1}\ d^{-1}$)
			2006	
E. angustifolia	0-P	2.30 a	0.029 b	0.002 b
	Low-P	2.21 a	0.040 ab	0.002 ab
	High-P	2.26 a	0.044 a	0.003 a
G. triacanthos	0-P	1.21 b	0.006 b	0.0005 b
	Low-P	1.65 a	0.009 b	0.0008 ab
	High-P	1.78 a	0.019 a	0.0010 a
R. pseudoacacia	0-P	1.34 c	0.012 b	0.002 b
	Low-P	1.53 b	0.019 ab	0.002 ab
	High-P	1.91 a	0.025 a	0.003 a
			2007	
E. angustifolia	0-P	0.55 a	0.024 b	0.004 a
	Low-P	0.53 a	0.037 a	0.004 a
	High-P	0.60 a	0.041 a	0.005 a
G. triacanthos	0-P	0.45a	0.011 c	0.002 b
	Low-P	0.21b	0.028 b	0.002 b
	High-P	0.33 ab	0.053 a	0.004 a
R. pseudoacacia	0-P	0.64 a	0.043 a	0.004 a
	Low-P	0.64 a	0.042 a	0.004 a
	High-P	0.54 b	0.051 a	0.005 a
			2008	
E. angustifolia	0-P	0.34 a	0.028 b	0.003 a
	Low-P	0.38 a	0.028 b	0.004 a
	High-P	0.35 a	0.038 a	0.005 a
G. triacanthos	0-P	0.12 a	0.028 a	0.001 b
	Low-P	0.20 a	0.029 a	0.002 a
	High-P	0.12 a	0.028 a	0.002 a
R. pseudoacacia	0-P	0.13 b	0.028 a	0.003 a
	Low-P	0.29 a	0.038 a	0.003 a
	High-P	0.25 a	0.039 a	0.003 a
		ANOVA, probability > F(=α)		
Year		<0.0001	<0.0001	<0.0001
Species		<0.0001	<0.0001	<0.0001
P treatment		<0.0001	<0.0001	<0.0001
Year*species		<0.0001	<0.0001	0.014
Year*P treatment		<0.0001	0.177	0.646
Species*P treatment		<0.0001	0.780	0.171

Note: Borderline significant 0.05<P<0.1; significant: 0.001<P<0.05; highly significant: P<0.001

Growth analyses based on nitrogen productivity

Nitrogen productivity (NP), which often is defined as the rate of weight increase per unit leaf N per time, is a useful indicator in growth analyses, as it depends on relative growth rates (Lambers et al., 1998). It is also considered an indicator of N investment by trees (vegetation) in foliage. This means that trees with higher NP have invested relatively more in foliage development than, for example, in roots or stems, and NP is therefore also a useful complement to R/S analyses (see section *Below and aboveground biomass partitioning*). The NP differed significantly among tree species over all years, *E. angustifolia* being superior. There was, however, no P effect on NP in *E. angustifolia* in 2006 and 2008, whilst the effect was inconsistent in *G. triacanthos* (Table 3.11). In contrast, a strong P effect on NP was observed in *R. pseudoacacia* in 2006, where high-P increased NP significantly compared to low-P and 0-P. In 2007 and 2008, NP with both P rates was higher than with 0-P (Table 3.11). This indicated that the increased growth of *R. pseudoacacia* was related to the increased N_2 fixation caused by P amendments.

Table 3.11: Nitrogen productivity (NP) of three species according to P treatments in 2006, 2007 and 2008. Within a column and each tree species, values followed by the same letter are not significantly different at *P*<0.05. Species overall means within a column followed by the same capital letter are not significantly different at *P*<0.05 according to Tukey post-hoc test

		2006	2007	2008
Species	P treatment		NP (mg g^{-1} day^{-1})	
E. angustifolia	0-P	6.94 a	2.37 ab	1.92 a
	Low-P	6.91 a	2.32 b	2.03 a
	High-P	6.93 a	2.41 a	1.95 a
Overall mean		6.93 A	2.37 A	1.97 A
G. triacanthos	0-P	5.88 b	2.21 a	0.95 b
	Low-P	6.50 a	1.40 b	1.58 a
	High-P	6.63 a	1.89 a	0.90 b
Overall mean		6.34 B	1.83 B	1.14 C
R. pseudoacacia	0-P	6.37 b	2.33 b	1.01 b
	Low-P	6.11 c	2.45 a	1.79 a
	High-P	6.76 a	2.45 a	1.64 a
Overall mean		6.55 B	2.41 A	1.48 B
			ANOVA, probability > F(=α)	
Year			<0.0001	
Species			<0.0001	
P treatment			<0.0001	
Time*species			<0.0001	
Time*P treatment			<0.0001	
Species*P treatment			0.031	

Note: Borderline significant 0.05<P<0.1; significant: 0.001<P<0.05; highly significant: P<0.001

In summary, the combined findings (Table 3.12) showed that compared to 0-P, high-P significantly increased N_2 fixation by the actinorhizal *E. angustifolia* and the leguminous species *R. pseudoacacia*. Total aboveground biomass production of *E. angustifolia* increased with high-P by more than 33%, whereas total biomass increased by more than 25%, but these differences were insignificant. Although total aboveground biomass increased with high-P by 70% and total biomass by 57% with *R. pseudoacacia*, these differences were also statistically insignificant. In comparison to 0-P, high-P significantly increased N concentrations in the foliage of *E. angustifolia*, and in the twigs and fine roots of *R. pseudoacacia*. Finally, with high-P, the UPR and NP of *R. pseudoacacia* increased significantly and also the relative growth rate in height (RGR_H) of *E. angustifolia.*

Table 3.12 Overall assessment of impact of high-P amendments compared to 0-P on selected biophysical and growth parameters after three years

Parameter	Unit	*Elaeagnus angustifolia*	*Gleditsia triacanthos*	*Robinia pseudoacacia*
N concentration in leaves	%	+	-	-
N concentration in twigs	%	-	-	+
N concentration in fine roots	%	-	+	+
Ndfa	%	+	na	+
Ndfa	kg ha^{-1}	+	na	+
Total biomass	t ha^{-1}	-	+	-
UPR	mg g^{-1} d^{-1}	-	-	+
RGR_H	mm mm^{-1} d^{-1}	+	-	-
RGR_D	mm mm^{-1} d^{-1}	-	+	-
NP	mg g^{-1} d^{-1}	-	-	+

Symbols indicate that the level of the parameter was statistically significant (+) or not (-) between high-P compared to 0-P treatments. na – not applicable

3.4 Discussion

3.4.1 Effect of P on N_2 fixation

Phosphorus is one of the minerals known to affect N_2 fixation and, along with N, P-deficiency is a major yield-limiting factor in many regions of the world (Pereira and Bliss, 1989). The access to P is essential for nodulation, N_2 fixation and plant growth (Marschner, 1986; Dommergues, 1995). Even small additions of P significantly increase plant nodule production as well as rates of N_2 fixation, and may even double the nitrogenease activity (Lynd et al., 1984). N_2 fixation by the *Frankia*-actinorhizal symbiosis is limited by low available P in the soil. Sanginga (1989), for instance, observed increased amounts of N_2 fixed by *Casuarina equisetifolia* after adding P to the P-deficient soil.

N_2 fixation (Ndfa in % and kg ha^{-1}) in *E. angustifolia*, which was estimated in this study both on a foliar and whole-tree basis, was the highest with high-P and the lowest with 0-P in all study years, which confirmed the effect of P on N_2 fixation also by this actinorhizal species. Moreover, a 4-year-old stand of *E. angustifolia* with high-P fixed between 64 (in the first year) and 807 kg N ha^{-1} (after three years) or an average 270 kg N ha^{-1} $year^{-1}$of atmospheric N_2 as estimated from the whole-tree ^{15}N abundance values (Table 3.7). When subtracting the values of a given year from those of the previous year, which yields the annual N_2 fixation amount, values ranged from 64–538 kg ha^{-1} $year^{-1}$ with high-P and from 25–342 kg ha^{-1} $year^{-1}$ with 0-P. The annual N_2 fixation values are higher than the lowest (24 kg ha^{-1} $year^{-1}$) and highest (514 kg ha^{-1} $year^{-1}$) values reported by Khamzina et al. (2009a) for *E. angustifolia* grown under the same environmental conditions, but without P amendments. The higher amounts of N_2 fixed could be attributed to the addition of P to the P-deficient soils. This conclusion is also supported by the annual N_2 fixed by *E. angustifolia* with 0-P, which ranged from 25-342 kg ha^{-1} $year^{-1}$, which corresponds to the earlier reported values of 24-514 kg ha^{-1} $year^{-1}$ (Khamzina et al., 2009a) for *E. angustifolia* without P fertilization. Also, the average amounts of N_2 fixed with high-P (about 270 kg N ha^{-1} $year^{-1}$) fall in the range previously estimated for this species. Compared to 0-P, *E. angustifolia* also fixed considerable amounts of N_2 with low-P, but the effect of this P amendment was inconsistent, and sometimes showed significantly higher and sometimes equal values compared to 0-P.

Since in low-P soils, the *Frankia-Casuarina* association required higher P levels than those needed for annual plant growth (Sanginga et al., 1989), it is likely that the low-P amounts applied were insufficient to obtain a consistent effect over the study years. In contrast, the amounts of P added with high-P gave consistently higher N_2 fixation rates. This may also be seen from the whole-tree based %Ndfa values estimated over the three-year period, where high-P additions increased %Ndfa by *E. angustifolia* from 85% (0-P) to 91%.

The *Rhizobium*-tree legume symbioses are reportedly able to fix between 43 and 581 kg of N ha^{-1} (Zahran, 1999), but most research findings refer to species of the genera *Acacia*, *Albizia* and *Cliricidia,* and the age of the stands is not always indicated. Quantitative estimates of BNF by the leguminous *R. pseudoacacia* under arid conditions such as those prevailing in the Khorezm region are lacking. A 4-year-old *R. pseudoacacia* stand fixed between 9 (after one year) and 155 kg N ha^{-1} (after three years) with high-P, whereas with 0-P the amount of N_2 fixed ranged between 2 (after one year) and 72 kg ha^{-1} (after three years). When subtracting the values of a given year from those of the previous year, the annual N_2 fixation by *R. pseudoacacia* with high-P ranged between 9 and 77 kg ha^{-1} $year^{-1}$ and with 0-P between 2 and 42 kg ha^{-1} $year^{-1}$. Danso et al. (1995) reported a N_2 fixation by 4-year-old *R. pseudoacacia* stands in Austria as high as 220 kg N ha^{-1}, measured with the ^{15}N isotope dilution method. This amount exceeds by far the total amount after three years with high-P in our study region. Such a difference may have been due to the elevated soil salinity at the study site (6-11 dS m^{-1}), which is known to suppress N_2 fixation (Galiana et al., 2004), or to the method of P application used. Yet, with high-P, N_2 fixation (kg ha^{-1}) increased by ca. 53% and %Ndfa from 76% (0-P) to 85%. This is in line with previous studies with leguminous species (Luyindula and Haque, 1992; Binkley et al., 2003). With high-P, the production potential of both the actinorhizal *E. angustifolia* and the leguminous species *R. pseudoacacia* could thus be exploited and increased on salt-affected croplands.

To obtain accurate estimates of the contribution of BNF it is necessary to consider all plant fractions rather than only individual tree fractions (Danso et al., 1995). Our results confirmed that foliar estimates of Ndfa underestimated the actual N_2 fixation ability of trees, especially when referring to the amounts of N_2 fixed.

A selection of appropriate non-fixing reference plants for accurate measurements of N_2 fixation with the ^{15}N abundance method is crucial. The reference species *G. triacanthos* used in this study depended purely on soil N for nutrition and had positive $\delta^{15}N$ values. According to Hogberg (1997), a minimum difference of 5% is required between the ^{15}N signals of the reference plants and N_2 fixers, which was met in case of *G. triacanthos*. The ^{15}N natural abundance values of the latter species differed significantly from the two N_2-fixing species examined, which exhibited negative $\delta^{15}N$. This is a common phenomenon that occurs in efficient N_2-fixing systems as argued by

Boddey et al. (2000). Hence, this significant difference in $\delta^{15}N$ of the N_2-fixers and non-fixer met the requirements of the ^{15}N abundance method. It is recommended by some authors to include, when possible, more than one reference species to increase the accuracy of quantification of N_2 fixation with the ^{15}N abundance method (Fried et al., 1983). However, *G. triacanthos* trees grew under the same conditions as the two N_2-fixing species and yielded the positive $\delta^{15}N$ values and N_2 fixation rates of both N_2-fixers, which were in line with the rates previously reported (Danso et al., 1992; Danso et al., 1995; Zahran, 1999; Khamzina et al., 2009a). We therefore consider the quantification of N_2 fixation against one species to be acceptable and trustworthy.

3.4.2 Phosphorus effect on biomass and growth rates

A sole application of high-P significantly increased the total biomass of the reference non-N-fixer *G. triacanthos.* Phosphorus fertilization of woody perennials is mostly recommended for commercially useful fruit species such as oilpalm (Taryo-Adiwigandaa et al., 2006), but in such cases mainly the impact of P amendments on fruit production has been reported. Oliet et al. (2005) recommended amendments of organic and/or inorganic fertilizers, including P, to nursery-grown tree saplings of the leguminous species *Acacia salicina* Lindl. to ease the well-known transplant stress. Especially on nutrient-poor soils in semiarid conditions, such additions increased long-term plantation establishment (Oliet et al., 2005). Despite the knowledge gap on the impact of P on non-fruit species, and certainly for the species home to Central Asia such as *G. triacanthos*, the higher P application significantly increased the total biomass of this species on the salt-affected croplands in semi-arid Uzbekistan over that of the control.

It has been reported that high N_2 fixation rates are mirrored also in higher biomass production of perennial crops although others reported no significant increase. Binkley et al. (2003), for instance, noted that despite having doubled the amount of N_2 fixed by seedlings of *Facaltaria moluccana* (Miquel) Barneby and Grimes, the biomass production increase was insignificant. Despite the substantial increases in the various biomass fractions, these increments were statistically insignificant for all trees examined in this experiment.

An ANOVA of growth revealed not only an increase in N_2 fixation with high-P, but also an increase in N concentrations in leaves of *E. angustifolia*, in twigs and fine roots of *R. pseudoacacia* (Table 3.5) and in the parameters favored by forestry physiologists such as UPR and NP of *R. pseudoacacia* and the relative growth rate in height (RGR_H) of *E. angustifolia.* But an ANOVA analysis did not detect a consistent effect of high-P on all species and over all years despite relatively large increases in total biomass in *E. angustifolia* and *R. pseudoacacia*. These inconsistencies could be in part explained by the relatively high standard deviations indicating that the number of replicates used or the number of trees harvested had been too small. A larger sample size can lead to increased accuracy of the parameters estimates (Steel and Torrie, 1980).

While the accuracy of some parameter estimates therefore may be debatable, an impact of P on parameters such as tree height in *E. angustifolia* was evident. Although the growth of trees in height precedes the growth in stem diameter, estimates of diameter growth often are considered more reliable than estimates of height growth because the measurement points for diameter growth monitoring are permanently marked on the stems. On the other hand, height growth was measured from the soil level to the highest vegetative point with a telescope measuring stick, which is also considered sufficiently accurate unless the trees are taller than the measuring stick (MacDicken et al., 1991).

There is a consensus that high growth rates of trees cannot be expected in a low-resource environment such as that at the experimental site (Table 3.1). But in such an environment, vegetation including tree species with high growth potential will grow faster than vegetation with a slow growth characteristics (Lambers et al., 1998). The RGR_H of *E. angustifolia* was generally higher for all P treatments and in all years compared to those of the other two species. Although with high-P the RGR_H of the species declined from 0.044 to 0.038 ($mm\ mm^{-1}\ d^{-1}$) over the three study years, the estimated RGR_H was still of the same order of magnitude as the RGR_H of *E. angustifolia* previously estimated for this species in the same study region in experiments without P fertilization (Lamers et al., 2006). On the other hand, the RGR_H of *E. angustifolia* with high-P was in general (much) higher in all years than the estimated RGR_H for other tree species in this arid environment, especially in the second study year of the above-mentioned study.

The outcomes of the present study along with the findings of Lamers et al. (2006) in the same study region underlined that P amendments increased N_2 fixation of both actinorhizal *E. angustifolia* and the leguminous *R. pseudoacacia* and can improve early (sapling) growth. However, the initial hypothesis that underpinned the experiments with P amendments was not entirely supported by the empirical evidence. Although the effect of high-P certainly increased N_2 fixation, these effects were not converted into consistent increased growth and biomass production. There are several possible explanations for these findings.

(1) The absolute amounts of P applied were insufficient, or the P applied was occluded by Ca, which is a common mineral in the soils of Central Asia (Pirahunov, 1977). When occluded as $Ca_3(PO_4)_2$, which is likely to occur under (slightly) alkaline conditions as monitored at the experimental site, P becomes unavailable to the trees or even could have led to Ca deficiency, which hence could have reduced growth. This perhaps could have occurred with low-P given the rather inconsistent findings with this treatment, but not with regard to the high-P amendments. The results with high-P showed, for instance, a significant increase in the UPR and NP of *R. pseudoacacia* and in the RGR_H of *E. angustifolia.*

(2) The P application method as practiced in the second season was less effective than assumed, which is also indicated by the increase in soil P in the second season (Table 3.1). Given that mineral P is less mobile in the soil than, for instance, N (Lambers et al., 2006), a localized P application (P-fertilizer placement) is generally recommended for tree fertilization (Darr and West, 1996). This is particularly necessary in arid climate conditions under which it has been monitored that roots preferably grow vertically (Khamzina et al., 2008). It may thus have been possible that the tree roots in the second growing season had less access to the P applied than assumed. On the other hand, the high N_2 fixation rates measured also in the second and third study years indicated that N_2 fixation had been boosted consistently with high-P but inconsistently with low-P.

(3) Perhaps N had not been the most limiting growth factor at this stage of tree growth on the salt-affected soils. Trees, just as other plants, show a functional response to limiting growth factors or to those in shortest supply (Lambers et al., 2006). Hence, plants invest in those parts that are needed to acquire the limited resource, and this

occurs at the expense of extending those plant components that are in need of the next resource in short supply. In other words, in the case of N-stress, plants would react by allocating more biomass to the roots (to acquire the N) than to the leaves (in need of N). Yet, the general decline in RSR in both *E. angustifolia* and *R. pseudoacacia* over the study years does not support this interpretation, thus indicating therefore the absence of N stress. Furthermore, the postulation that N was not in short supply was supported also by the absence of typical N-deficiency symptoms such as chlorotic foliar starting from the older leaves. Given all the parameters observed and measured, it is still unclear which other parameter could have been in short supply, if at all.

(4) A probable explanation is that the tree species examined in this study behaved similar to many fruit species with respect to storing and mobilizing N. In general, annual and perennial herbaceous and woody species satisfy their N demand for growth and development by an uptake of N as nitrate, ammonium, or organic N (Gessler et al., 1998; Nasholm et al., 1998), or by the translocation of N sources stored as proteins and amino acids (Wetzel et al., 1989; Sagisaka, 1993; Stepien et al., 1994), particularly in the perennial organs such as coarse roots, stem and bark, or trunk (Millard, 1996; Dong et al., 2002; Frak et al., 2002). Whereas N storage in deciduous woody species predominantly occurs at the end of a growing season in autumn, while during springtime N is remobilized (Tagliavini and Millard, 2005) to satisfy the N demand for the development of newly growing shoots and leaves without relying on soil N in the rooting zone during this growth stage (Dong et al., 2002). Thus trees, in contrast to annual crops, may rely on the remobilization of the internal N sources, which may have been accumulated during previous years. Given that the highest N concentrations at the end of the growing seasons were found in the leaves and woody, perennial organs (coarse and fine roots) irrespective of years, species and P applications, N remobilization could have satisfied the N demand at the onset of the growing seasons.

In order to elucidate the possible reason or combination of reasons for the inconsistencies, the first explanation could be eliminated by an application of higher P rates, the second with a different P application method, whereas the third would demand an intensive monitoring of various parameters presently excluded as they were beyond the scope of this study. The fourth reason would demand an intensive monitoring and analyses of the xylem sap at different periods of the year, but this was also beyond the scope of this study.

Despite the absence of a consistent impact of P amendments, the ability of both *E. angustifolia* and *R. pseudoacacia* to be self-sufficient in N with high-P on the impoverished and saline soils of the croplands in arid Uzbekistan was increased, as previously postulated (Khamzina et al., 2009a), and high-P increased various growth parameters. This renders indeed both species suitable for the afforestation of such soils in the irrigated croplands of Uzbekistan.

3.5 Conclusions

Nitrogen-fixing trees play an important role in the rehabilitation of degraded soils and in promoting soil fertility of managed landscapes. The results showed that also the actinorhizal *E. angustifolia,* on which relatively few studies have focused thus far, as well as the leguminous *R. pseudoacacia*, on which no information was found, maintained their ability to fix N_2 in the impoverished and saline soils prevailing in the irrigated areas of Uzbekistan. This was substantiated by the overall percentage of N_2 derived from atmosphere (%Ndfa) of 87% and 82% for *E. angustifolia* and *R. pseudoacacia*, respectively, over a three-year period.

The growth of N_2-fixing trees is often limited by a low supply of soil P, mainly because P deficiency may limit N_2 fixation by these trees. An alleviation of P soil deficiencies through P fertilization increased N_2 fixation by *E. angustifolia* with 81% when compared to the control without P amendments. With a P application of about 90 kg P ha^{-1} (high-P), *R. pseudoacacia* doubled N_2 fixation in comparison to trees without P applications (0-P). The amounts of N_2 fixed with high-P ranged from 64-807 kg ha^{-1} and 9-155 kg ha^{-1} in *E. angustifolia* and *R. pseudoacacia*, respectively.

Despite a significant effect of P on N_2 fixation, this was not mimicked in a significant increase in biomass as analyzed by a standard ANOVA. But that P additions not only increased N_2 fixation but also boosted growth of both N_2 fixers was revealed by the analyses of various growth rate parameters (e.g., increase in height and stem diameter) that are recommended particularly for analyzing the growth performance of perennial woody vegetation (Lambers et al., 1998). Hence, when relative growth rates were used in the analyses rather than absolute growth rates, a significant effect of high-P was observed on the UPR and NP for the leguminous tree species *R. pseudoacacia* and on the RGR_H of the actinorhizal species *E. angustifolia*. This confirms that research on forestry should include such an approach to the analysis.

The findings thus confirmed not only that both the actinorhizal N_2-fixing *E. angustifolia* and the leguminous species *R. pseudoacacia* have the potential to be self-sufficient in N on the low-fertile and degraded croplands in arid Uzbekistan, but also that even small additions of P (as low as 32 g P tree^{-1}) could increase N_2 fixation and consequently the productivity of plantations. When taking into account the N_2- fixing ability and the amounts of N, which the species examined added to the plant-soil system, *E. angustifolia* along with *R. pseudoacacia* are good candidates for the rehabilitation of the salt-affected croplands to the benefit of people and the environment. Furthermore, P fertilization bolstered the foliage N content of in particular *E. angustifolia* and indirectly therefore also the feed quality (see Chapters 6, 7).

The symbiotic performance of actinorhizal *E. angustifolia* and leguminous *R. pseudoacacia* was estimated with the ^{15}N natural abundance method, but it is often recommended to include more than one reference species. In addition, this method has the reputation of being the most reliable among other methods for quantifying N_2 fixation. Yet, for accurate estimations, the collection of all tree fractions is necessary, which is often problematic in the case of older trees in open field trials (Boddey et al., 2000). Given these potential limitations for quantifying N_2 fixation by *E. angustifolia*, a lysimeter experiment was concurrently conducted, which allowed harvesting the entire trees and comparing and verifying the N_2 fixation rates with those by *E. angustifolia* in the field trial. In addition, two reference trees were used to increase the accuracy of the methods used in the lysimeter trial (Chapter 4), which was also designed to appraise four different methods for quantifying N_2 fixation (Chapter 5). Due to logistical reasons, these verification studies did not include *R. pseudoacacia,* for which few reference values for Central Asian regions were available, either.

4 QUANTIFICATION OF SYMBIOTIC NITROGEN FIXATION BY *ELAEAGNUS ANGUSTIFOLIA* L. ON SALT-AFFECTED IRRIGATED CROPLANDS USING TWO ^{15}N ISOTOPIC METHODS

4.1 Introduction

Land degradation has become a global concern in the 21st century owing to its adverse economic, social and environmental impacts. Worldwide, the areas vulnerable to land degradation amount to about 33% of the global land surface (WMO, 2005), of which the irrigated lowlands of Central Asia, including Uzbekistan, are considered as very severely affected (Lal, 2000). The on-going land degradation in Uzbekistan is caused by soil salinization, rising saline groundwater tables, frequent droughts, and human-induced water erosion (Saigal, 2003; Ibrakhimov et al., 2007). In Khorezm, an administrative district of Uzbekistan that is representative for the irrigated lowlands in Central Asia, about 20% of the irrigated land has already been classified with a 'bonitet' (soil quality) index of 40 and lower, which is the threshold for marginal land (Martius et al., 2004). Responsible are not only high soil salinity levels, saline and shallow groundwater tables, but also deficiencies in soil nitrogen (N), the latter being considered the most limiting factor for crop growth and biomass production in the Khorezm region (Kienzler et al., 2007).

Nitrogen-fixing trees (NFTs) can rehabilitate degraded soils (Dommergues, 1995; Masutha et al., 1997). Hence, the afforestation with multipurpose trees and shrubs, in particular the actinorhizal *Elaeagnus angustifolia* L. (Russian olive), has been proposed to make economic use of degraded croplands within the irrigated areas of the Khorezm region (Khamzina et al., 2006; Lamers et al., 2006). The established small-scale forests with this salt-tolerant, fast-growing, multipurpose tree species can provide useful products such as timber, fuelwood (Lamers and Khamzina, 2008) and leaf fodder (Djumaeva et al., 2009). Yet little is known about the ecological benefits of this species, such as its contribution through N_2 fixation to soil fertility. Ample evidence exists on the atmospheric N_2 fixed by leguminous crops, but much less information is available on NFTs, particularly on species grown in the irrigated croplands of Central Asia. According to a review of studies in tropical regions (Danso et al., 1992), NFTs may fix as much as 43-581 kg of N ha^{-1} annually. The annual amount of N_2 fixed by *E.*

angustifolia in Uzbekistan varies from 24-514 kg ha^{-1} $year^{-1}$ (Khamzina et al., 2009a). However, the trees studied were in an open field trial, and the estimates were partly based on the analyses of leaves alone and partly on the total N in the trees, which perhaps are the reasons for the reported high inter-annual variations.

Variations of N_2-fixation by trees are associated not only with the species, age and density of plantations, and soil conditions, but also with the wide variety of methods used to quantify biological nitrogen fixation (BNF) (Boddey et al., 1995). The acetylene reduction assay (Hardy et al., 1968; Roskoski, 1981) and the ureide assay methods (Herridge et al., 1994; Peoples et al., 1996) are based on indirect, qualitative, yield-dependent criteria. The total-N-difference method (Gauthier et al., 1985; Ndoye and Dreyfus, 1988a) requires reference species that acquire the same amount of N from the soil as the N_2-fixer (Unkovich et al., 2008; Khamzina et al., 2009a). For the estimation of BNF in trees, ^{15}N isotope methods are widely used, since they provide yield-independent and time-integrated estimates of the percentage of N derived from atmospheric N_2 (%Ndfa) (Peoples et al., 2002). The ^{15}N isotope techniques depend on differences in isotopic composition of the sources of N available for growth such as soil, groundwater and fertilizer N, as well as atmospheric N_2 (Bergensen and Turner, 1983). There are two approaches: The natural ^{15}N-natural-abundance technique (^{15}NNAT) exploits the slight ^{15}N enrichment of available soil N to differentiate it from atmospheric N, whereas the ^{15}N-enrichment technique (^{15}NET) amplifies the enrichment of soil N by adding ^{15}N-enriched amendments such as fertilizers. The ^{15}NET has proved to be an effective and straightforward approach for measuring BNF by trees in plantations and under field conditions (Baker et al., 1992b; Boddey et al., 1995b).

^{15}N methodologies require the comparison of the N_2-fixer with non-fixing reference plants grown in a soil fertilized with similar ^{15}N backgrounds, enriched or not. The addition of only small amounts of ^{15}N materials to the N_2-fixing plant is suggested to prevent inhibition of N_2 fixation, but these amounts must still be sufficient to ensure a proper growth of the reference plants. The A-value (AV) method deals specifically with cases where, unlike the ^{15}NET, higher doses of ^{15}N are applied to the non-fixer than to the N_2-fixer (Fried and Broeshart, 1975). The ^{15}NET requires also that all species absorb their N from the same N sources, which makes the application of the enriched fertilizer, the uniform distribution of the amendments and the selection of satisfactory reference plants crucial (Witty, 1983; Danso et al., 1992; Chalk and Ladha, 1999). Although the ^{15}NET has experienced widespread field application, the violation of the various key assumptions has led to considerable errors in the estimation of BNF by trees (Anhar, 2005). This holds true in particular for large, deep-rooting trees growing in soils that were not uniformly labeled with ^{15}N, causing a differential extraction from various soil depths with differently labeled N pools. When using the ^{15}NET in an open system, labeled fertilizer can be lost during salt leaching or irrigation (Sanginga et al., 1989; Boddey et al., 2000). Also, the volatilization of ^{15}N-enriched fertilizer applied as an aqueous solution to the soil surface turned out to be a source of considerable errors (Sanginga et al., 1996). Furthermore, to estimate the total amount of N_2 fixed, an assessment of the total dry matter yield as well as total N in the plant is needed, which is time and resource consuming to obtain in perennial vegetation (Boddey et al., 1995; Boddey et al., 2000).

The aim was therefore: (1) to quantify the whole-tree %Ndfa (percentage of N derived from atmospheric N_2) and total N_2 fixed by *E. angustifolia* using the ^{15}NET and AV methods, and (2) to compare these two methods.

4.2 Materials and methods

4.2.1 Description of the study sites

In 2007 and 2008, a lysimeter trial was conducted on the experimental site of the Urgench State University located at 41°33′ N latitude, 60°36′ E longitude at an altitude of 101 m asl in Khorezm, Uzbekistan. During the growing seasons, the mean air temperature was approximately 17°C with minimum and maximum daily temperatures ranging from -8°C to 43°C, respectively. The mean annual rainfall of 100 mm fell mostly outside the growing season. The mean relative air humidity varied between 26% and 86% over both years.

4.2.2 Experimental design

Preparation of the lysimeters: In 2007, twelve and in 2008 twenty closed-bottom steel lysimeters of 120 cm (depth) x 50 mm (diameter) were painted on the inside and outside and placed into soil openings of 1 m depth so that ca. 20 cm remained above the soil surface. The distance between lysimeters within a row was 1 m and the distance between rows was 1.75 m. Gravel (34 kg) was thoroughly washed and filled into each lysimeter as a bottom layer to accommodate drainage. A tin plate with a diameter of 50 cm was cut at both sides in a herringbone pattern and placed on top of the gravel followed by cloth and paper, which avoided a mix-up of the soil particles with the gravel. A polyethylene pipe of 125 cm length and 25 mm diameter was placed into a hole made at a right angle to the edges of the tin plate and close to the rim of the lysimeter to assure an equal distribution of the irrigation water that was applied from below. To settle the soil inside the lysimeters, irrigation water was added via the polyethylene pipe during three consecutive days prior to planting. To prevent leaching losses of ^{15}N, closed lysimeters were used.

Selection and planting of trees: To quantify BNF by *E. angustifolia*, the tree species Honey locust *(Gleditsia triacanthos* L.*)* and Siberian elm (*Ulmus pumila* L.*)* were selected as references. Saplings were grown in a nursery of ca. 0.5 ha size on a gleyic calcaric arenosol. Prior to seedbed preparation, this area was ploughed, chiseled, and leveled. Trees were fertilized according to the recommendations in Uzbekistan (MAU, 1982), i.e., N was applied at the equivalent of 120 kg N ha^{-1} in three doses: 60 kg N ha^{-1} before soil preparation and 30 kg N ha^{-1} each at 3 and 4 months after seeding. Phosphorus (P) was applied at the equivalent of 90 kg P ha^{-1} as 60 kg P ha^{-1} before soil preparation and 30 kg P ha^{-1} three months after seeding. Fertilizers were applied at the bottom of the 15 cm deep irrigation furrows and incorporated manually. The saplings were irrigated at least 10 times during each growing season. Shortly before transplanting the one- and two-year old saplings into the lysimeters, 10 of each species in 2007 and 14 of each in 2008 were selected for average size (diameter and height of the stem), extracted, cleaned from the soil, and transplanted bare-rooted into the lysimeters at a density of one sapling per lysimeter. The saplings were planted in three replications. Each lysimeter was selected randomly. From the remaining saplings, the initial mean overbark diameter was measured at 10 cm above the soil surface with a calliper, the height measured with a measuring tape, and the initial dry mass was determined following oven-drying. The 2-year-old saplings in 2008 were larger than the 1-year-olds used in 2007 (Table 4.1).

Soil characteristics: The soil used to fill the lysimeters in 2007 and 2008 was extracted according to the existing soil layers from the Yangibazar Research Station of the

Urgench State University: a first layer of 0-25 cm, a second of 25-65 cm and a third of 65-110 cm. The excavated soil layers were air-dried separately, thoroughly sieved (6 mm) to remove stones and unwanted debris, and placed into the lysimeters according to the soil horizon strata in the field. The average weight of the soil in the lysimeters was 260 kg constituting a total volume of 0.24 m^3.

Table 4.1: Means (n=6) of stem diameter, height and dry matter of three tree species at the onset of 2007 and 2008. Values in brackets are standard deviations of the means

Year	2007			2008		
Species	*Elaeagnus angustifolia*	*Gleditsia triacanthos*	*Ulmus pumila*	*Elaeagnus angustifolia*	*Gleditsia triacanthos*	*Ulmus pumila*
Diameter (mm)	6.9 (±1.7)	6.2 (±1.4)	5.5 (±0.5)	11.3 (±0.4)	10.0 (±2.7)	8.0 (±0.5)
Height (cm)	78.8 (±18.5)	69.3 (±4.7)	51.3 (±9.8)	111.6 (±16.7)	89.4 (±15.7)	108.3 (±15.0)
Dry matter (g)	28.3 (±11.4)	16.4 (±6.0)	7.7 (±1.6)	59.1 (±7.0)	30.7 (±3.3)	20.6 (±2.3)

The soil was predominantly of a silt-loam texture (Khamzina, 2006). Physical and chemical soil characteristics in 2007 are given in Table 4.2. In 2008 (data not shown), soil characteristics were similar to those in the previous year except for the chloride, sodium and sulphate concentrations, which in the top 25 cm were 2.5 times higher. Since the tree roots were positioned below this layer, an influence on the overall tree growth was not expected. Soil fertility in both years was poor as evidenced by the low soil organic matter and NPK concentrations. Electrical conductivity (EC) over the 1 m soil layer was 9.8 dS m^{-1}, indicating severe soil salinity according to Abrol et al. (1988). The soil-organic carbon concentration was analyzed according to Tyurin (1975), and total N was determined using the Kjeldahl method. Available P was measured with a colorimeter in an ammonium carbonate extract (Protasov, 1977).

Application of ^{15}N fertilizer ($^{15}NH_4{}^{15}NO_3$): The soil placed in the lysimeters was labeled with ^{15}N-enriched ammonium nitrate (35% N, 5 atom % ^{15}N excess), applied twice during the 2007 and 2008 growing seasons. The first fertilization occurred 46 days after transplanting (DaT) the one-year-old saplings in 2007 and two-year-old saplings in 2008 from the nursery into the lysimeters. In 2007, the ^{15}N-labeled fertilizer was applied to all trees at the rate of 4 g N per lysimeter (10.384 g ammonium nitrate), corresponding to 20 kg N ha^{-1}, which was three times lower than the recommended annual rate of 60 kg N ha^{-1} for trees in Uzbekistan (MAU, 1982). Since the initial soil-N content was low (Table 4.2), and since the rate of 4 g N per lysimeter could have been insufficient for the growth of the larger reference trees in 2008 (Table 4.1), a second treatment was added. In addition to the 4 g N, in 2008 12 g N (31.151 g ammonium nitrate) per lysimeter was used for comparison, which equaled the recommended rate of 60 kg N ha^{-1} for trees. The treatments with 4 g N contained 0.575 g ^{15}N per lysimeter, whilst the 12 g N treatments contained 1.725 g ^{15}N. Given the potential inhibitory effect of high rates of N fertilization on N_2 fixation in leguminous plants (Sanginga et al., 1996), the rate of 12 g N was not added to the N_2-fixing *E. angustifolia*. Because of the different amounts of ^{15}N applied to the N_2-fixer and non-fixer, the AV method was used to estimate the N_2 fixation amount (Fried and Broeshart, 1975).

Table 4.2: Physical and chemical soil characteristics in lysimeters (0-110 cm) in 2007. Values in brackets are standard deviations of the means

Parameter	Soil depth (cm)		
	0-25	25-65	65-110
Bulk density (g cm^{-3})	1.2 (±0.0)	1.5 (±0.0)	1.5 (±0.0)
pH	7.8 (±0.1)	7.9 (±0.1)	7.8 (±0.2)
Dry residue (%)	0.2 (±0.1)	0.1 (±0.0)	0.1 (±0.0)
Soil organic matter (%)	0.9 (±0.2)	0.7 (±0.2)	0.4 (±0.1)
Total N (mg kg^{-1})	0.26 (±0.02)	0.22 (±0.01)	0.16 (±0.09)
P_2O_5 (mg kg^{-1})	17.6 (±9.5)	14.1 (±8.8)	8.3 (±3.2)
K_2O (mg kg^{-1})	120 (±31)	101 (±23)	92 (±25)
Cl^- (cmol kg^{-1})	1.9 (±0.2)	0.3 (±0.0)	0.5 (±0.3)
Na^+ (cmol kg^{-1})	3.3 (±1.4)	0.6 (±0.1)	0.5 (±0.1)
SO_4^{-2} (cmol kg^{-1})	6.2 (±2.3)	0.7 (±0.1)	0.7 (±0.1)

In both years, the ^{15}N fertilizer was applied twice, 50% in May and 50% in July. The fertilizer was weighed with a precision balance to the nearest mg and added to a plastic bottle, which contained one liter of distilled water. The bottles were thoroughly shaken for two hours to ensure that the fertilizer was completely dissolved and mixed evenly. Every lysimeter received one liter of this solution, which was added through the polyethylene pipe. To attain an equal distribution of the ^{15}N throughout the soil profile in the lysimeter, 10 l of irrigation water were added through the same pipe immediately after applying the fertilizer solution (Figure 4.1).

Management: Saplings were irrigated fortnightly with 15 l of desilted irrigation water. In July, the hottest month of the year, this amount was increased to 20 l. The soil surface in the lysimeters was continuously cleared of weeds to avoid undesirable competition for ^{15}N. The weeds were placed on top of the soil in each lysimeter and after air-drying incorporated into the soil.

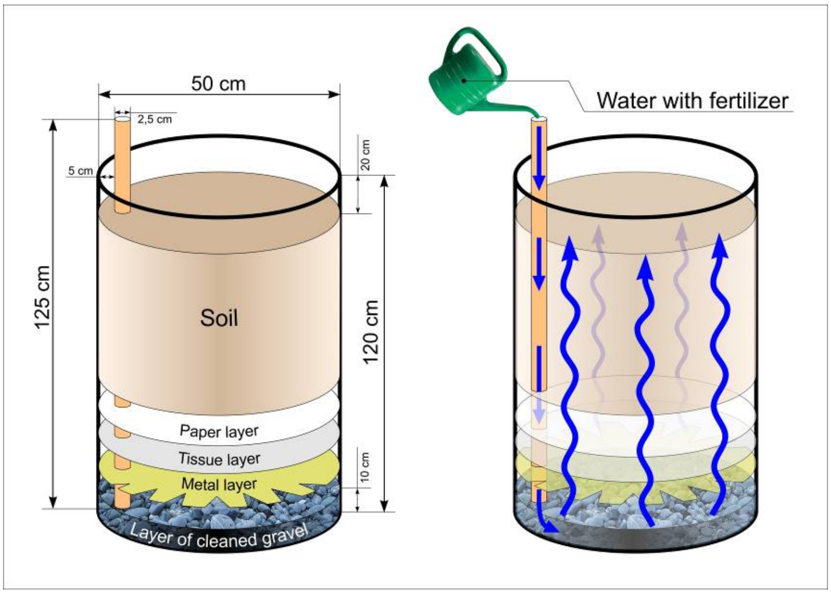

Figure 4.1: Illustration of the application procedure of fertilizers with water leading to a homogenous distribution of the ^{15}N enriched fertilizer over the soil profile in the lysimeter

4.2.3 Plant and soil measurements

Harvest and sample preparation: At 135 DaT, 12 trees (3 species x 4 replicates) in 2007 and 20 trees (3 species x 4 replicates for 4 g N, and 2 species x 4 replicates for 12 g N) in 2008 were harvested for above- and belowground dry matter (DM) determination. Trees were cut at the level of the soil surface, aerial fractions were separated into leaves, twigs (∅<2 mm) and stem (∅>2 mm), and belowground fractions into coarse (∅>2 mm) and fine roots (∅<2 mm). The fresh mass of each tree fraction was weighed immediately after harvest with a portable electronic scale to the nearest 0.01 gram. Above- and belowground parts were placed in separate paper bags and oven -dried at 50 ºC for 72 hours until constant weight. These dried samples were ground to pass through a 2-mm sieve and analyzed for %N and $\%^{15}N$ using an ANCA mass spectrometer (SL/20-20, SerCon, UK).

Soil sampling: The soil in the lysimeters was sampled in 0.2 m layers down to 1 m depth. Soil samples were air-dried, finely ground in a mill and analyzed for %N and $\%^{15}N$ using the ANCA mass spectrometer (SL/20-20, SerCon, UK).

4.2.4 BNF estimates

The percentage of N derived from atmospheric N_2 (%Ndfa): %Ndfa of *E. angustifolia* using the ^{15}NET was calculated from whole-tree atom $\%^{15}N$ excess according to the equation (FAO/IAEA, 2001):

$$\%\,Nda = \left(1 - \frac{Atom\ \%\ ^{15}N\ excess\ fixer}{Atom\ \%\ ^{15}N\ excess\ non\ fixer}\right) \cdot 100 \qquad (4.1)$$

where 'atom $\%^{15}N$ excess' is the value of the samples after subtracting $\%^{15}N$ atmosphere (standard, 0.3663 $\%^{15}N$). This was calculated for both reference species, i.e., 'atom % ^{15}N non-fixer' was either based on *G. triacanthos* or on *U. pumila*.

For the estimation of %Ndfa of *E. angustifolia* with the AV method (Fried and Broeshart, 1975), the equation as outlined by Hardarson et al. (1991) was used:

$$\%\,Ndfa = 100\left(1 - \frac{\%Ndff\ fixer}{n \cdot \%Ndff\ non\ fixer}\right) + \%Ndff\ fixer\left(\frac{1}{n} - 1\right) \qquad (4.2)$$

where %Ndff fixer is the percentage N derived from the fertilizer applied to the fixer, %Nddf non fixer is the percentage N derived from fertilizer by the non-fixer, and *n* is the amount of fertilizer applied to the N_2-fixer divided by the amount of fertilizer applied to the non-fixer.

Total amount of N_2 fixed: The amount of N_2 fixed by *E. angustifolia,* using both reference species, was calculated according to:

$$Amount\ of\ N_2\ fixed = \frac{\%Ndfa \cdot total\ N\ in\ fixer}{100} \quad (4.3)$$

The DM production and N accumulation of *E. angustifolia* was converted from g tree^{-1} as observed in one lysimeter to kg ha^{-1} by assuming a density of 5,000 trees ha^{-1}.

4.2.5 Statistical analyses

Weighted means are presented with ±1 standard deviation. One-way analysis of variance (ANOVA) was used to analyze differences in atom % ^{15}N excess and $\%N_{tot}$ in trees and soil as well as the differences in above- and belowground biomass among the tree species. When significant (P<0.05) differences were found, the Tukey post-hoc test was used to compare individual treatment means. The student's *t*-test, while assuming equal variances, was used to examine differences between foliar and whole-tree atom % ^{15}N excess, %Ndfa and total fixed N_2 values. In the 2008 data set, outliers occurred in the third replicate of both reference tree species. To avoid distortions in data interpretation, these outliers were replaced by the mean value of the first and second replicate. Statistical analyses were performed with SPSS 15.0.

4.3 Results

4.3.1 Total dry matter production and tissue nitrogen content in 2007 and 2008

The homogeneity in sapling size, as observed at the onset of both study seasons (Table 4.1), allowed the comparison of the species as a response to their growth in the lysimeters. In 2007, *G. triacanthos* produced significantly lower total DM (ca. 73 g tree^{-1}) than *E. angustifolia* and *U. pumila*, which produced 134 and 125 g tree^{-1}, respectively, in the 4 g N treatment (Figure 4.1). In the same fertilizer treatment in 2008, two-year-old *E. angustifolia* saplings produced significantly more DM (223 g tree^{-1}) than both reference species. In contrast, the DM of 59 g tree^{-1} of the two-year-old *G. triacanthos* in 2008 was 19% lower than that of the one-year-old *G. triacanthos* saplings in 2007. To a lesser extent, the same was observed for two-year-old *U. pumila* saplings, which in 2008 had a 22% lower total DM production than in 2007.

For accurate estimates of N_2 fixation with the ^{15}NET method, it is essential that the reference and N_2-fixing plants use soil N of identical ^{15}N enrichment (Boddey et al., 2000). To achieve that, among others root biomass and its distribution over the soil horizons of the N_2-fixing and reference plants need to be similar so that the same soil volume is explored. In 2007, this requirement was met (Figure 4.1). In 2008, the root DM of *G. triacanthos* (17 g tree^{-1}) and *U. pumila* (28 g tree^{-1}) was nearly 4- and 2-fold lower, respectively, than that of *E. angustifolia* (66 g tree^{-1}). In addition, the root distribution of *G. triacanthos* was restricted mainly to the top 45-cm soil horizon, whilst the roots of *E. angustifolia* were distributed over the entire 98 cm depth of the lysimeter. In contrast, with 12 g N, in 2008 the two-year-old *G. triacanthos* and *U. pumila* trees produced a root biomass similar to that of *E. angustifolia*. In addition, the root distribution of *G. triacanthos* and *U. pumila* trees of 77 and 83 cm, respectively, was more similar to that of *E. angustifolia*; total root DM was respectively 75% and 56% higher compared to the root growth of the same species in the 4 g N treatment.

The reduced overall tree production and root DM production of *G. triacanthos*, and to a lesser extent *U. pumila* in the 4 g N treatment, was thus caused by a N deficiency, which had not impacted the N_2-fixer *E. angustifolia*. Since the criterion that reference plants and N_2-fixer should assimilate the same ratio of labeled N was not met as assumed for the 4 g N treatments in 2008 (because of large difference in root biomass and differences in root distribution), the findings of the 4 g N treatments in 2008 were unsuitable for the ^{15}NET method. Instead, in the second year, the AV method was used to assess the BNF, thus only the 12 g N treatment was used.

The highest N accumulation in *E. angustifolia* occurred in the leaves (ca. 4.8 kg ha^{-1} in 2007 and ca. 6.1 kg ha^{-1} in 2008). Additionally, all other fractions in *E. angustifolia* accumulated ca 3.3 kg ha^{-1} in 2007 and ca 15.2 kg ha^{-1} in 2008, which confirms that not just the leaves but the whole tree needs to be considered for assessing the total amount of N_2 fixed (Boddey et al., 2000).

4.3.2 Atom %^{15}N excess and %N_{tot} in plants and soil

In 2007 and 2008, the atom %^{15}N excess values were significantly (P<0.05) lower in *E. angustifolia* than in the reference trees when all tree fractions were pooled (Table 4.2). In both years, *U. pumila* had significantly higher %^{15}N excess values compared to *E.*

angustifolia and *G. triacanthos*. *Elaeagnus angustifolia* accumulated significantly more N compared to both reference species in 2007 and 2008.

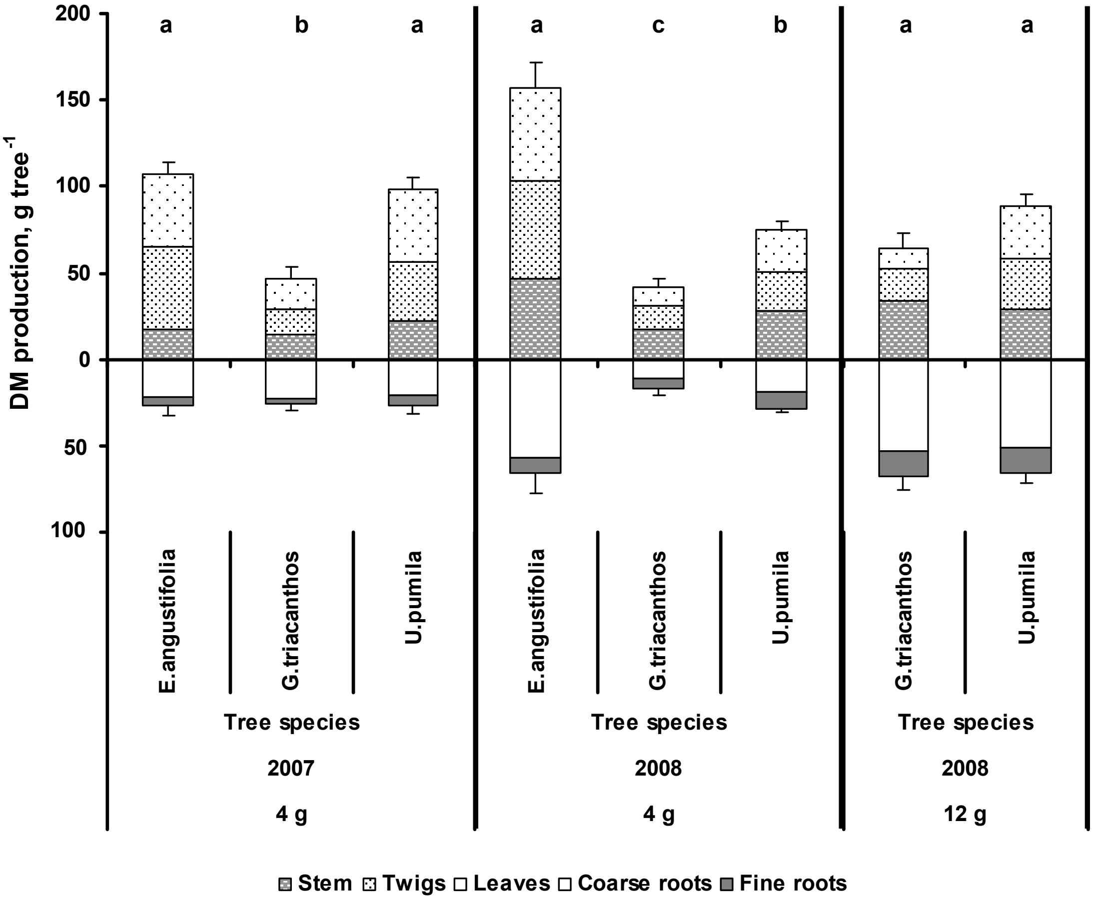

Figure 4.1: Above- and belowground dry matter production in 2007 and 2008 of three tree species and according to nitrogen (N) fertilizer applied. Vertical bars represent standard deviations. The values with the same letter are not significantly different for species in each year and fertilizer treatment

The differences in atom $\%^{15}N$ excess in soil were insignificant between soil depths horizons at harvest in 2007 (Figure 4.2a) and 2008 (data not shown), irrespective of N rates applied, thus confirming the effectiveness of the enrichment application method used. The $\%N_{tot}$, on the other hand, was highest in the upper 0-60 cm and lower in the 60-100 cm horizon in 2007 (data not shown); most importantly, however, no significant differences in atom $\%^{15}N$ excess were found between the different soil depths in 2008 (Figure 4.2b).

Table 4.2: Atom $\%^{15}N$ and $\%N_{tot}$ combined over all tree fractions of N_2-fixing and two reference tree species in 2007 with ^{15}NET and in 2008 with AV method. Values within a column followed by the same letter are not significantly different at $P<0.05$ according to the Tukey post-hoc test; values in brackets are standard deviations of the means

Tree species	2007	2008
	Atom% ^{15}N excess	
Elaeagnus angustifolia	1.194 (±0.592) c	0.501 (±0.114) c
Gleditsia triacanthos	2.029 (±0.917) b	1.507 (±0.113) b
Ulmus pumila	3.171 (±0.355) a	2.044 (±0.116) a
	N_{tot} (%)	
Elaeagnus angustifolia	1.5 (±0.9) a	1.9 (±0.3) a
Gleditsia triacanthos	1.0 (±0.5) b	1.4 (±0.4) b
Ulmus pumila	1.0 (±0.3) b	1.4 (±0.4) b

4.3.3 Proportion of N derived from atmospheric N_2 (%Ndfa) in *E. angustifolia*

Using the ^{15}NET on the 2007 total biomass dataset, the %Ndfa of *E. angustifolia* amounted to 79% and 68%, with *U. pumila* and *G. triacanthos* as reference species, respectively. In 2008, the %Ndfa of *E. angustifolia* obtained with the AV method was 80% and 68%, respectively, when relating to the same reference tree species in 12 g N treatment.

a) 2007 **b) 2008**

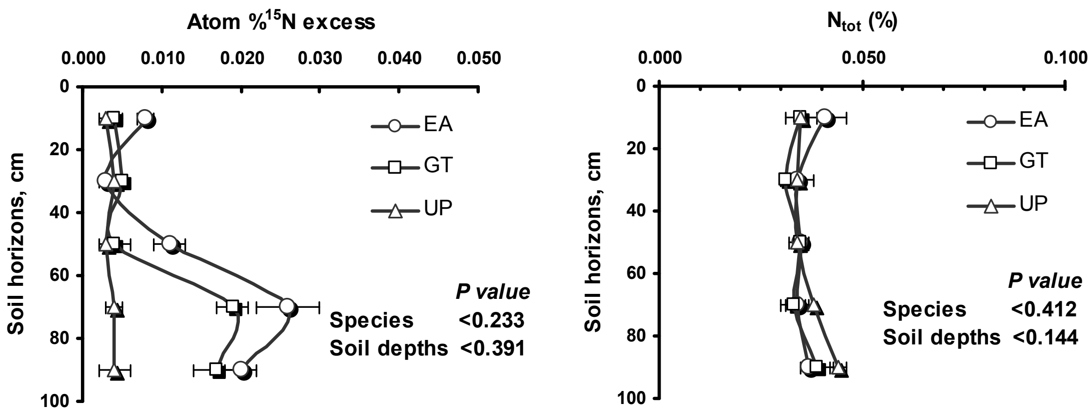

Figure 4.2 a/b: End of season atom $\%^{15}N$ excess in soil in 2007 (a) and $\%N_{tot}$ means in soil in 2008 (b) at different depths in lysimeters for N_2-fixing *E. angustifolia* and reference species *G. triacanthos* and *U. pumila*. Horizontal bars represent standard deviations

The differences between foliar and whole-tree atom $\%^{15}N$ of *E. angustifolia,* estimated with the student's *t*-test, were insignificant in 2007 ($P(T<=t)$=0.919) and 2008 ($P(T<=t)$=0.766). The same was true for the differences in foliar and whole-tree atom %^{15}N of the reference species *U. pumila* (in 2007: *P(T<=t)*=0.532; in 2008: *P(T<=t)*=0.814) and *G. triacanthos* (in 2007: *P(T<=t)*=0.528 and in 2008: *P(T<=t)*=0.134). Similarly, the differences in foliar %Ndfa and whole-tree %Ndfa values of *E. angustifolia* in 2007 with the ^{15}NET were not significant compared to *G. triacanthos* (*P(T<=t)*=0.921) nor to *U. pumila* (*P(T<t)*=0.919). Furthermore, the different amounts in foliar %Ndfa and whole-tree %Ndfa values of *E. angustifolia* in 2008 as determined with the AV method were insignificant against both reference species (*G. triacanthos*: *P(T<=t)*=0.766; *U. pumila*: *P(T<=t)*=0.766).

4.3.4 Total amount of N_2 fixed

The amount of N_2 fixed by *E. angustifolia* with the ^{15}NET in 2007 was 5.49 (±1.30) kg ha^{-1} when referenced against *G. triacanthos* and 6.41 (±1.50) kg ha^{-1} when referenced against *U. pumila.* In both cases, the bulk (60%) of the fixed N_2 was located in the leaves. According to the AV method, the total amount of N_2 fixed in 2008 was higher than in 2007 due to the higher growth rate of the trees. The estimated BNF differed according to the reference trees. The total amount of N_2 fixed by *E. angustifolia* was 14.57 (±1.31) kg ha^{-1} when referenced against *G. triacanthos* and 16.04 (±1.45) kg ha^{-1} when referenced against *U. pumila*.

The estimates of the total amount of N_2 fixed reveal a significant difference between foliar and whole-tree N_2 fixed in both years when related to *U. pumila* (*P(T<=t)*=0.014 in 2007 and *P(T<=t)*=0.001 in 2008) as well as when related to *G. triacanthos* (*P(T<=t)*=0.001 in 2007 and *P(T<=t)*=0.0003 in 2008), suggesting that the whole-tree N_2 fixed provided more accurate estimates of the total N contribution to the system.

4.4 Discussion

4.4.1 Dry matter production and suitability of ^{15}N methods

The amount of 4 g N applied to the one-year-old saplings apparently sufficed to avoid N deficiency for the growth of both reference species in 2007. Indeed, there were no visual symptoms of N stress such as yellow leaves or premature leaf drop. In 2008, the overall growth of the two-year-old *G. triacanthos* and *U. pumila* trees with the 4 g N application was lower compared to that of both species with a 12 g N supplement. The

4 g N application did not constrain the growth and DM production of the N_2-fixing *E. angustifolia* in either year. Both reference species with the 4 g N treatment in 2008 were N-limited owing to their larger size and thus increased N demand. Under such conditions the ^{15}NET method should not be applied. The AV approach is in such cases more reliable, as it was developed to deal with different ^{15}N application levels (Fried and Broeshart, 1975).

4.4.2 Accuracy of measurements of N_2 fixation

Lysimeters will not accommodate trees for a longer period without constraining root growth. To bypass this potential hurdle, in the present study the trees in the lysimeters were compared each season but at two different growth periods using one- and two-year-old saplings. On a tree basis, two-year-old *E. angustifolia* trees in an open field trial in Uzbekistan fixed amounts of N_2 (about 4 g tree^{-1}) comparable to those measured in this lysimeter study (Khamzina et al., 2009a). The difficulties associated with harvesting larger trees for obtaining total DM (Boddey et al., 2000) could thus be avoided by the use of lysimeters. Not only was it possible to estimate the total dry matter of the trees, the method also allowed assessing the amount of N_2-fixed based on both individual fractions and the entire tree. Moreover, the simple technique of using a polyethylene pipe in a lysimeter through which water and labeled ^{15}N fertilizer could be supplied from below effectively ensured a uniform distribution of the ^{15}N fertilizer throughout the soil profile.

The selection and number of appropriate reference plants is critical for estimating BNF, since these factors affect the accuracy of the ^{15}N isotopic methods (Sanginga et al., 1989a; Chalk and Ladha, 1999; Boddey et al., 2000b). In the absence of appropriate non-fixing isolines, the use of several uninoculated reference species has become a common procedure (e.g. Ndoye and Dreyfus, 1988; Sanginga et al., 1989a; Danso and Kumarasinghe, 1990). The non-nodulating legume *G. triacanthos* and the non-N-fixing *U. pumila* used in this study fulfilled the main requirement of reference species (Fried et al., 1983) as both relied solely on soil N. The use of lysimeters avoided the possible cross contamination that may occur between N_2-fixer and non-fixer in open-field trials (Sanginga et al., 1989). Given the insignificant differences in the distribution of atom % ^{15}N excess across all soil layers in the lysimeters (Figure 4.2a), the distribution can thus be considered as homogeneous. Based on this, the pattern of N assimilation by all tree species must have been similar, thus meeting the chief criteria for applying ^{15}N isotopic methods (Fried et al., 1983; Witty, 1983).

4.4.3 %Ndfa by E. angustifolia

The %Ndfa by *E. angustifolia* depended on the reference species against which it was measured. When referenced against *U. pumila* and *G. triacanthos* in 2007, the %Ndfa by *E. angustifolia* was 79% and 68%, respectively. The AV method used in 2008 yielded %Ndfa of 80% and 68% with the same reference trees, similar to the results of 2007. According to FAO/IAEA (2001), %Ndfa estimates higher than 70% provide results with less error, whereas for %Ndfa values of 30% and below, the accuracy of the ^{15}NET is considered insufficient. Since our findings ranged from 68-79% in 2007 and from 68-80% in 2008, the values obtained fell within the acceptable margin of errors.

Parrotta et al. (1994) postulated that a well-targeted foliar sampling could be as accurate to determine tissue ^{15}N-enrichment as whole-tree sampling. In such cases, a random sampling of the foliage over the entire canopy is required. Since differences in values of atom %^{15}N excess between foliar and whole-tree in the lysimeters were insignificant in both 2007 and 2008, the present findings confirm those of Parrota et al. (1994) that leaves can be taken for the estimation of %Ndfa.

4.4.4 Amounts of N_2 derived from BNF

We observed highly significant differences between the estimated amounts of foliar and whole-tree N_2 fixed by *E. angustifolia* in both years. This underscores the necessity to use the total biomass including roots and nodules as previously suggested to obtain accurate estimations of the total N_2 fixed (Sanginga et al., 1996; Khan et al., 2002).

The values obtained with the one- and two-year-old *E. angustifolia* saplings in this lysimeter study were relatively low when compared to the values reported for different two-year-old NFTs (Sanginga et al., 1989; Parrotta et al., 1994). In 2007 and 2008, the amount of N_2 fixed by the one-year-old *E. angustifolia* in the lysimeters amounted to ca. 6 and 16 kg ha^{-1} year^{-1}, respectively, when *U. pumila* was used for comparison and ca. 5 and 15 kg ha^{-1} year^{-1} for *G. triacanthos*. Even with these small amounts, the N_2-fixing *E. angustifolia* has the potential to be self-sufficient in N when planted in the salt-affected irrigated croplands in Central Asia as was previously concluded in open-field studies and with older trees of the same species (Khamzina et al., 2009a).

4.5 Conclusions

N_2 fixation by *E. angustifolia* was quantified in lysimeters for saline soils, as these predominate in the irrigated croplands of Central Asia. The ^{15}NET shows a high accuracy and reliability with one-year-old trees when using low doses of enriched ^{15}N fertilizer. However, for the two-year-old trees this amount of ^{15}N fertilizer was suspected insufficient for a proper growth of the reference species, especially given the low initial soil fertility. The application of higher amounts of ^{15}N fertilizer to the reference trees overcame this limitation, and BNF estimates were possible using the AV method. This method yielded similar outcomes as ^{15}NET and is thus considered accurate and reliable to deal with different ^{15}N application levels.

The amount of N_2 fixed by one- and two-year-old *E. angustifolia* saplings grown in lysimeters was low compared to that reported previously (Khamzina et al., 2009a) and in the open field (see section 3.3.3), but still realistic given the salinity levels of the soil used in the lysimeters. The amounts of N_2 fixed confirm that *E. angustifolia* can be used as part of a larger set of strategies to exploit salt-affected irrigated croplands of Central Asia.

The lysimeter trail was designed also for comparing four different N_2 fixation quantification methods with the aim to identify the most suitable one while taking into account not only the accuracy of the N_2 fixation quantification findings, but also potential costs (see Chapter 5).

5 AN APPRAISAL OF FOUR METHODS FOR QUANTIFYING THE END-OF-SEASON N_2 FIXATION RATES OF *E. ANGUSTIFOLIA* L. GROWN IN LYSIMETERS

5.1 Introduction

With 6.8 billion, the global population has now more than doubled since 1945 and is projected to reach about 9 billion by 2050 (US Bureau of census, 2010). To sustain the production of food, feed, fiber and fuel for the growing population, sufficient soil nitrogen (N) must be accessible as this mineral is a major component of protein and chlorophyll (Madakadze et al., 1999) and thus indispensable for the growth of natural and domestic vegetation. Although the highest N amounts are fixed in the earth's crust (Mengel and Kirkby, 1982), the sole source of soil N is atmospheric diatomic N (Barbarick, 1996).

Before the manufacturing of N fertilizers through the Haber-Bosch process, atmospheric N_2 had entered the soil through lightning and precipitation, crop remnants and its decomposition, manure, or biological nitrogen fixation (BNF) by annual and perennial vegetation. The global amounts of N_2 fixed through BNF reached about $140x10^6$ metric tons per year from agricultural, forest and non-agricultural lands combined, which was twice as much as the total N_2 fixation by non-biological processes (Deacon, 2003).

Since the discovery of the BNF process by Beijerinck and others between 1895 and 1904 (Chung and Ferris, 1996), researchers have aimed at understanding the essence of the BNF process and quantifying the amount of N added to the soil-plant system. A wide range of techniques has been explored with annual and perennial vegetation and the methods presently available for measuring BNF are based on (1) increment in N yield and plant growth; (2) N balance; (3) acetylene reduction (based on the enzyme nitrogenase activity) and (4) the use of stable isotopes of N (see e.g. Hardy et al., 1968; Fried and Broeshart, 1975; Fried and Middelboe, 1977; Bergensen and Turner, 1983).

For the time-integrated measurements of N_2 fixation and the quantification of the percentage of N derived from the atmosphere (%Ndfa) under field conditions, the total N difference (ND) (Munroe and Davies, 1974), ^{15}N enrichment (^{15}NE) (McAuliffe et al., 1958), ^{15}N natural abundance (^{15}NA) (Shearer and Kohl, 1986), and A-value (AV) (Fried and Broeshart, 1975) methods have been considered most suitable. But irrespective of the availability of the numerous methods for measuring BNF, no method could be singled out yet as being the 'correct' way to quantify N_2 fixation by plants (e.g. Peoples et al., 2002; Azam and Farooq, 2003). The amount of N_2 fixed depends on environmental factors, including soil type, soil nutritional status, plant species and varieties, water availability and temperature (Ledgard and Steele, 1992), which may explain the wide variation in reported findings for the amount of N_2 fixed and the %Ndfa of species. But variations are also caused by the methods used for measuring N_2 fixation, which is particularly true for N_2-fixing tree (NFT) species (Boddey et al., 1995).

In general, BNF methods require a detectable and measurable increase in the total N content in the soil-plant system. However, because of relatively low additions to an already big pool of N, obtaining realistic estimates of BNF are often cumbersome especially with perennial vegetation (Boddey et al., 1995) due to difficulties in quantifying the total dry matter production of trees and bushes. Their perennial nature, large size with often extended root systems, and the large tree-to-tree and nodulation variations within a tree stand render the system unsuitable for simple sampling techniques (Boddey et al., 1995). Also inter-seasonal and inter-annual differences in nodulation have led to the large variations in reported values (Wong et al., 1989; Fownes and Anderson, 1991). Before selecting any method, clear goals and objectives for measuring BNF must be formulated. Equally important is an assessment of the financial resources and materials constraints. For instance, the use of ^{15}N to quantify BNF may very well give the most accurate quantification, but is associated with high costs for the ^{15}N fertilizer and sample analyses (Boddey et al., 1995). Therefore, the use of more than one reference species and/or the simultaneous use of several methods are recommended, as they not only will complement each other but also can increase the accuracy of the quantification.

The treatments in the lysimeter trial to quantify the BNF of actinorhizal *Elaeagnus angustifolia* L. by using the ^{15}NE and AV methods (see Chapter 4), included also a control without ^{15}N-labeled fertilizer applications. This control treatment allowed the comparison of the findings of the two isotopic methods with the total ND and ^{15}NA method. In all four methods, *Gleditsia triacanthos* L. and *Ulmus pumila* L. served as reference. The aim of this component was thus to estimate and compare the end-season N_2 fixation rates of *E. angustifolia* in four different ways.

5.2 Materials and methods

During 2008, a lysimeter trial was conducted on the premises of the Urgench State University in the Khorezm region of Uzbekistan (41°33´ N latitude, 60°36´ E longitude, 101 m altitude). From leaf flushing in March to harvest in October 2008, the mean air temperature was approximately 17°C. The mean annual rainfall of 100 mm fell mostly outside the study period.

For the experiment, 24 closed-bottom steel lysimeters of 120 (depth) x 50 cm (diameter) were assigned completely randomly to positions in a 20 by 20 m sized bare field with a spacing of 1 m by 1.75 m distance in the soil at 1 m depth (ca. 20 cm remained thus above the soil surface). Each lysimeter was filled first with 34 kg gravel, which was covered with a tin plate of 50 cm in diameter, and then by cloth and paper. A polyethylene pipe (length: 125 cm length; diameter 25 mm) was positioned into a hole in the tin plate to assure an equal distribution of the irrigation water, which was applied from below.

5.2.1 Treatments: species and fertilizer application

The lysimeter trial was designed as a two-factorial experiment. To quantify the BNF by *E. angustifolia,* two non-N-fixing tree species served as reference: Honey locust *(Gleditsia triacanthos* L.*)* and Siberian elm (*Ulmus pumila* L.*)*. In addition, three N fertilizer applications were used: (i) a control with no fertilization and (ii) 4 g N per lysimeter (10.384 g ammonium nitrate corresponding to 20 kg N ha^{-1}), which was applied as ^{15}N enriched ammonium nitrate ($^{15}NH_4\,^{15}NO_3$, 35% N, 5 atom % ^{15}N excess). While this application allowed employing the ^{15}NE method for quantifying N_2 fixation, given the initial low soil N content (see Chapter 4, Table 3.1) this rate was assumed to be insufficient to sustain the growth of the two-year-old reference trees. Therefore a third treatment was added: 12 g N per lysimeter (31.151 g ammonium nitrate, corresponding to the recommended rate of 60 kg N ha^{-1} for trees in the study region (MAU, 1982). Since high rates of N fertilization may delay N_2 fixation in leguminous plants (Sanginga et al., 1996), the rate of 12 g N was not added to N_2-fixing *E. angustifolia*. The AV method (Fried and Broeshart, 1975) could thus be used to calculate the N_2 fixation rate in this case.

The design covered thus two N treatments for the N_2-fixing *E. angustifolia* and three N treatments for the two reference species *G. triacanthos* and *U. pumila*. Each of these treatments was replicated three times. Each of the 24 lysimeters hosted one two-year-old nursery-grown seedling.

The ^{15}N fertilizer was applied as a split application: half in May (46 days after transplanting (DAT) and half in July (120 DAT). Prior to these applications, the fertilizer was weighed with a precision balance to the nearest mg and added to a plastic bottle with 1 liter of distilled water. The bottles were thoroughly shaken for two hours to obtain an even mix, which was then added through the polyethylene pipe to each lysimeter. To enhance the uniform distribution of the ^{15}N applied over the soil profile in the lysimeter, 10 l of irrigation water were added through the same pipe immediately after applying the N-fertilizer solution. The detailed description is given in Chapter 4.

5.2.2 Soil characteristics

The silt-loam texture soil (Khamzina, 2006) in the lysimeters stemmed from the Yangibazar Research Station of the State Urgench University. The soil was extracted according to its field layers that appeared at 0-25 cm, 25-65 cm 65-110 cm, air-dried, thoroughly sieved (6 mm) and placed into the lysimeters according to the same soil strata in the field. The average soil weight in the lysimeters was ca. 260 kg. The soil organic matter content (see Chapter 4, Table 4.2), as analyzed according to Tyurin (1975), was low as were the contents of N, determined by the Kjeldahl method, and available P as measured with a colorimeter in an ammonium carbonate extract (Protasov, 1977).

5.2.3 Plant and soil measurements

At 135 DaT, above- and below-ground dry matter (DM) was determined of all 24 trees (N-fixer: 1 species x 2 treatments x 3 replicates; reference: 2 species x 3 treatments x 3 replicates). Following their cutting at the soil surface, the fresh weight of the leaves, twigs (∅ < 2 mm), stems (∅ >2 mm), coarse (∅ >2 mm) and fine roots (∅ < 2 mm) was immediately determined with a portable electronic scale to the nearest 0.01 gram. All fractions were separately placed in paper bags, oven-dried at 50ºC for 72 hours until constant weight, ground to pass through a 2 mm sieve and analyzed for %N and %^{15}N

using an ANCA mass spectrometer (SL/20-20, SerCon, UK). The DM production and N accumulation of *E. angustifolia* was converted from g tree^{-1} in one lysimeter to kg ha^{-1} by assuming a density of 5,000 trees ha^{-1}, which was close to the stand density of the same species in a concurrently conducted field experiment.

At harvest, the soil in the lysimeters was sampled in 0.2 m layers and these samples were air-dried, finely ground in a mill and then analyzed for total N and %^{15}N using the ANCA mass spectrometer (SL/20-20, SerCon, UK).

5.2.4 Data processing and methods used for BNF quantification

Following the chemical analyses of all tree fractions (leaves, twigs, stem, fine and coarse roots), the relevant data sets were used to estimate the BNF capacity of *E. angustifolia* by the total ND, ^{15}NA, ^{15}NE and AV methods referenced against *G. triacanthos* and *U. pumila*.

Total N difference method

The amount of N_2 fixed by *E. angustifolia* according to the total ND method was estimated as:

$$N_2\,fixed = (N\,yield_{fix}) - (N\,yield_{ref}) \tag{5.1}$$

where N yield $_{fix}$ is the total N content of the N_2-fixer *E. angustifolia* and N yield $_{ref}$ is the total N content of the reference non-N-fixing species, i.e. *G. triacanthos* and *U. pumila*.

^{15}N natural abundance technique

The %Ndfa by *E. angustifolia* was quantified according to Shearer and Kohl (1986) (for details, see Chapter 3, Equation 3.1).

^{15}N isotope-based quantification methods

The %Ndfa of *E. angustifolia* with the ^{15}N enrichment method was computed according to (FAO/IAEA, 2001) (for details, see Chapter 4, Equation 4.1). For the estimation of %Ndfa of *E. angustifolia* with the AV method (Fried and Broeshart, 1975), the equation as outlined by Hardarson et al. (1991) was used (for details, see Chapter 4, Equation 4.2). The amount of N_2 fixed by *E. angustifolia,* using both reference species, was calculated for the last three methods according to Equation 4.3 (see Chapter 4).

5.2.5 Statistical analyses

With the analysis of variance (ANOVA), the differences were analyzed in a whole-tree based N_2 fixation (%Ndfa, kg ha^{-1}) measured with ND, ^{15}NA, ^{15}NE atom and AV methods as well as between the above- and below-ground biomass of three tree species under different methods. When significant (P<0.05) differences were found, the Tukey post-hoc test was used to compare individual treatment means. Statistical analyses were performed with SPSS 15.0.

5.3 Results and discussion

The challenge to accurately quantify the N_2 fixation (%Ndfa, kg ha^{-1}) of a tree has motivated researchers worldwide to experiment with various methods (Table 5.2).

5.3.1 The total nitrogen difference method (ND)

The amount (kg ha^{-1}) of the whole-tree based N_2 fixation by *E. angustifolia* according to the total ND method was about 14 kg N_2 ha^{-1} when referenced against both *G. triacanthos* and *U. pumila* (Table 5.3). The differences of N_2 fixed between the ND and the AV method was ca. 1 kg ha^{-1} for *G. triacanthos* and 2 kg ha^{-1} for *U. pumila* (Table 5.3) but these differences were statistically not significant from the AV and nor the ^{15}NE (only for *U. pumila*) methods. This indicated on the one hand the suitability of the ND method in lysimeter studies since it involved much lower costs for enriched fertilizers and laboratory equipment. But on the other hand, the difference of 1 to 3 kg ha^{-1} compared to three methods equaled to 7-14%, which could be unacceptable when aiming at the quantification of the N_2 fixation for large areas.

It is assumed that the N present in the reference plants represents the amount of soil mineral N that was available for plant growth during the observation period. While assuming further that the N_2-fixing species assimilates the same amount of soil N as the non-fixer, the difference in N content between the two species is assumed to be due to N_2 fixation. The ND method is thus a simple, straightforward method that quantifies BNF at low costs. It is argued, however, that this method gives reliable estimates of N_2 fixation mainly under conditions of low soil N and in case of large differences in N yield between the N_2-fixer and the non-N-fixing control (e.g. LaRue and Patterson, 1981). The precondition that the N_2-fixing species and reference plants have similar root uptake activity and patterns, may be questioned when the latter is a completely different species (Rennie and Rennie, 1983). When the total ND method lacks precision, it is primarily due to the widely divergent N acquisition pattern of the N_2-fixer and non-fixers (Azam and Farooq, 2003).

Our lysimeter results confirmed those of Broadbent (1982), Rennie (1984), and Mueller and Thorup-Kristensen (2002), showing that the BNF quantification by the total ND method can be in good agreement with the ^{15}N-determined values for N_2 fixation. This can be explained not only by the similar root traits of *E. angustifolia* and the reference plants, in particular of *U. pumila* (see Chapter 4), but also by the accuracy in determining soil N in lysimeters and the entire dry matter mass of the trees, and by avoiding soil N losses, which is often a complicating factor in open field experiments (Boddey et al., 2000).

Very recent research results suggested that the traditional total ND procedure can be improved by accounting for both inter-specific root interactions and soil N loss for the estimation of BNF in legume/non-legume intercropping systems (Yu et al., 2010). However, the suggested improved total ND method requires that the amount of soil mineral N (Nmin) available to the N_2-fixer and the reference plants is the same at the same time (Herridge et al., 2008). This requirement could not be verified with the data sets from the present lysimeter study.

5.3.2 The ^{15}N abundance method (^{15}NA)

Following their extended review of methods to quantify the BNF by trees and shrubs, Boddey et al. (1995; 2000) and also many others (e.g. Baker et al., 1992; Chalk and Ladha, 1999) argued that the ^{15}NE and ^{15}NA methods, when correctly applied and used, provided the most accurate amount of symbiotic N_2 fixation. The comparison, however, showed that according to the ^{15}NA method, the %Ndfa by *E. angustifolia* varied between 0.4 and 2.9% when referenced against *G. triacanthos* and *U. pumila,*

Table 5.2: Review of methods used for quantification of N_2 fixation by perennial vegetation

Method	Advantages	Disadvantages	Suitability for perennial crops	Estimated accuracy	Source
Total N-difference (ND)	• Simple, low-cost • Does not require the addition of ^{15}N fertilizer	• Requires large soil and plant sampling, • Multi year analyses preferable • Differences in root morphology and rooting depth of N_2- and non-N fixer	Modestly	Partly	Boddey, 1987; Ladha et al. 1993; Chalk, 1998
^{15}N natural abundance (^{15}NA),	• Provides a possibility to monitor N_2 fixation in any location where N and non-N-fixer are present • Does not involve the addition of ^{15}N fertilizer	Needed are • *B*-value, • Suitable reference plants, • Whole tree dry matter, • Expensive sample analyses	Highly suitable	High	Boddey et al. 1995; Wong et al. 1989; Fownes and Anderson, 1991
^{15}N enrichment (^{15}NE)	• Provides a time-integrated estimate of N_2 fixation over one or more growing seasons • Distinguish between soil, fertilizer and fixed N_2	• Labeled soil N needs uniform distribution over the rooting zone, • Needs to be stable with time • High cost of ^{15}N fertilizer and sample analyses • Requires reference plants that mimic the growth characteristics of the N-fixer	Highly suitable	High	Witty, 1983; Peoples et al. 1989; Danso et al. 1992; Parrotta et al. 1994; Sanginga et al. 1995
A-value (AV)	• Provides a time-integrated estimate of N_2 fixation over one or more growing seasons • Distinguish between soil, fertilizer and fixed N_2	• Labeled soil N needs uniform distribution over the rooting zone, • Needs to be stable with time • High cost of ^{15}N fertilizer and sample analyses • Requires reference plants, which mimics the growth characteristics of the N_2-fixer	Modestly	High	Fried et al. 1983; Fried and Broeshart, 1975

respectively, and that the amount varied between 0.008 when referenced against *G. triacanthos* and 0.05 kg ha^{-1} when compared to *U. pumila* (Table 5.3). The estimates of the whole-tree based N_2 fixation by *E. angustifolia*, were thus both in terms of %Ndfa and quantity (kg ha^{-1}) not only much lower than those of the ND, ^{15}NE and AV methods and irrespective from the reference species *G. triacanthos* and *U. pumila*, but also indicated hardly any N_2 fixation according to the ^{15}NA method.

Table 5.3: Estimates of the whole-tree based end-of-season N_2 fixation (Ndfa) by *E. angustifolia* (%Ndfa, kg ha^{-1}) with the total N-difference (ND), ^{15}N natural abundance (^{15}NA), ^{15}N enrichment (^{15}NE), and A-value methods (AV) referenced against *G. triacanthos* and *U. pumila.* Values between brackets are standard deviations of the means (n=3). Means in the same column followed by the same letter are not significantly different at *P*<0.05 according to the Tukey post-hoc test

Method	*G. triacanthos*		*U. pumila*	
	N_2 fixation (Ndfa)			
	%	kg ha^{-1}	%	kg ha^{-1}
ND	na	14 (±4) a	na	14 (±3) a
^{15}NA	0.4 (±0.0) b	0.008 (±0.01) b	2.9 (±0.1) c	0.05 (±0.0) b
^{15}NE	-0.3 (±0.0) b	-0.08 (±03) b	58 (±3) b	12 (±2) a
AV	68 (±5) a	15 (±2) a	80 (±2) a	16 (±2) a

***na**- not available*

The ability of the actinorhizal *E. angustifolia* to produce nodules in saline soils of the study region Khorezm and consequently fix N_2 was suggested already by Khamzina et al., (2006). More recent findings demonstrated that the N_2-fixing *E. angustifolia* had the potential to be self-sufficient in N when grown in the salt-affected irrigated croplands in this region, fixing between 24 kg ha^{-1} in the first year and 514 kg ha^{-1} in the third year, depending, among others, on the age and density of the *E. angustifolia* plantations (Khamzina et al., 2009a). Given that the *E. angustifolia* saplings in the present lysimeters grew under similar bio-physical conditions, originated from the same nursery and grew on the soil stemming from the same site as previously examined (Khamzina et al., 2009a), it is unrealistic to assume that the two-year-old *E. angustifolia* saplings in our study did not fix N_2 as suggested by the results of the ^{15}NA method. This argument is supported by the findings of the other three methods used in this lysimeter study, indicating that *E. angustifolia* fixed between 12 and 16 kg N_2 ha^{-1} while the %Ndfa varied between 58% and 80%.

The ^{15}NA method is applicable when the ^{15}N of the N derived from BNF and that from the soil by the N_2-fixer can be separated (Boddey et al., 2000). Only then BNF can be estimated as the differences in atom %^{15}N of soil available N and atmospheric N_2. This does not require the use of costly fertilizers as needed with the ^{15}NE and AV methods (Table 5.4).

Table 5.4: Financial resources spent (in USD) for quantifying N_2 fixation with total nitrogen difference (TND), ^{15}N natural abundance (^{15}NA), ^{15}N enrichment method (^{15}NE) and A-value (AV) techniques in 2008

Method		Cost of enriched ^{15}N fertilizer	No. of samples	Chemical analyses: Plant ^{15}N	Soil ^{15}N	Total N	Total amount spent (in USD)
TND		-	3			9	27
^{15}NA		-	3	19			57
^{15}NE	4 g	40*	3	----------19--------			2280
	12 g	120*	3	----------19--------			6840
AV	4 g	40*	3	----------19---------			2280
	12 g	120*	3	----------19---------			6840

** The price for 1 g of ^{15}N fertilizer was 10 USD at the study year 2008*

The estimation of BNF by the ^{15}NA method requires, however, knowledge about the *B* value (Equation 5.2), which is the amount of N_2 fixed by the N_2-fixer in N-free conditions (Shearer and Kohl, 1986). In the absence of an accurate *B* value (in ‰) for *E. angustifolia* species indigenous to the study region Khorezm, we used a whole-tree *B* value of -1.41 ‰ which had been determined for actinorhizal tree species (Domenach et al., 1989; Tjepkema et al., 2000). However, while using this reported *B* value, our estimates of N_2 fixation with the ^{15}NA method showed only very low N_2 fixation. To obtain similar BNF quantities as estimated with the other methods used, the *B* value would have needed to be around 0, which is a value reported by several other authors (Boddey et al., 2000). Boddey et al. (2000) ascribed this discrepancy to the fact that various reported *B* values have been derived from single-tree fractions rather than the obligatory examination of the entire set of fractions, including roots, shoots and nodules. Since our lysimeter results were in line with the previous findings (Khamzina et al., 2009a), indicating the potential of *E. angustifolia* to fix atmospheric N_2, it seems very likely that the choice of the *B* value from the literature leads to erroneous results. The findings of the ^{15}NA method should thus be interpreted with caution as long as the *B* value has not been specifically determined for the local *E. angustifolia.*

5.3.3 The ^{15}N enrichment (^{15}NE) and the A-value (AV) methods

To the best of our knowledge, the ^{15}NE and the AV methods had not been used for the quantification of the amount of diatomic N fixed by *E. angustifolia* in the arid regions and under the salt-affected croplands in the irrigated systems of Central Asia. This is likely caused by the practical difficulties involved in quantifying N_2 fixation by NFTs due to their perennial nature and large size (Boddey et al., 1995) as well as the high costs of fertilizer and plant tissue analyses. The ^{15}NE method involves an enrichment of the soil by adding equal amounts of ^{15}N enriched fertilizer to the N_2-fixing and non-N-fixing reference species. The differences in derived ^{15}N between the N_2-fixer and non-fixer(s) are then used to calculate the amounts of N_2 fixed.

A distinct advantage of the ^{15}N isotope dilution technique to estimate the amount of BNF is its ability to give an integrated estimate of N_2 fixation over a growing season or even beyond. More importantly, it can, in contrast to all other methods, distinguish between soil, fertilizer and fixed N_2 in field-grown crops (Danso, 1988). Although the ^{15}NE method is considered one of the most robust methods to quantify N_2 fixation in perennial vegetation (e.g. Boddey et al., 2000), its use is often restricted by the high costs of the ^{15}N fertilizer and sample analyses (Table 5.4). Moreover, both the AV-method and ^{15}NE methods involve not only the use of costly ^{15}N fertilizer (Table 5.4) but require reference plants, which ideally should mimic the root-growth characteristics of the N_2-fixer. The latter could be achieved in particular in the presence of a non-nodulating host, which is not always available.

An additional consideration with the ^{15}NE method is the dilemma that high doses of ^{15}N fertilizer may potentially depress N_2 fixation by the N_2-fixer (Fried and Broeshart, 1975), whilst low doses may be insufficient to support the good growth of the non-fixing reference species, especially in soils with low N contents. Although in such cases different amounts of enriched N fertilizer can be applied to the N_2-fixer and non-fixer, this contradicts one of the key assumptions of the ^{15}NE method. Consequently, to bypass this potential N dilemma, a slightly higher dose is applied to the non-fixer, whereas the N_2-fixer receives a lower quantity. The AV method was designed to deal with such cases (Fried and Broeshart, 1975) and was applied in this study in the case of *G. triacanthos* as a reference plant. When this species was used as a reference to estimate the fixation of *E. angustifolia* with the ^{15}NE method without the application of extra N to the trees, the %Ndfa and N_2 fixed (kg ha^{-1}) values were negative (Table 5.3), meaning that the low amount of ^{15}N fertilizer applied did not suffice for its proper growth (Table 5.5) and the rooting patterns between the N_2-fixer and reference plants diverged (see Chapter 4).

Table 5.5: Total dry matter production in 2007 and 2008 according to the nitrogen (N) fertilizer applied. Values between brackets are standard deviations of the means (n=3). The values with the same letter are not significantly different for species within each year and fertilizer treatment. Means in the same column followed by the same letter are not significantly different at $P<0.05$ according to the Tukey post-hoc test

Tree species	2007 4 g	2008 4 g	2008 12 g
Elaeagnus angustifolia	134 (±7) a	233 (±21) a	na
Gleditsia triacanthos	73 (±5) b	59 (±8) c	131 (±9) a
Ulmus pumila	125 (±6) a	103 (±5) b	154 (±7) a

na *- not available*

5.4 Conclusions

The comparison of the end-of-season N_2 fixation rates of *E. angustifolia* with four different procedures referenced against *U. pumila* grown in lysimeters demonstrated no significant differences in the whole-tree based N_2 fixation measured with the ND, ^{15}NE and AV methods. In case of the second reference *G. triacanthos*, there was an agreement between the ND and AV techniques only. Based on previous results showing that *U. pumila* was the most suitable of the two reference species because of its almost similar root growth characteristics as N_2-fixing *E. angustifolia* (see Chapter 4), and given that the AV method allowed the addition of small amounts of enriched ^{15}N without inhibiting tree growth in the lysimeters (see Chapter 4), it is very likely that the AV method with *U. pumila* as a reference gave the most accurate findings. Yet, in case financial, human and material resources are restricted, the use of the total ND method in combination with lysimeters can be a feasible alternative, but then the facilities for dry matter and total N determination need to be warranted. As long as the *B* value necessary for the employ of the ^{15}NA is not determined for the symbiotic associations of soil organisms and the N_2-fixing *E. angustifolia* grown on salt-affected croplands in arid regions, the findings of this method should be interpreted with caution.

It is not only beneficial to boost N_2 fixation but also to become aware of the leaf N dynamics of trees and plantations during the growing season, especially because N_2 fixation tends to increase foliar N content, such as for *E. angustifolia* (see Chapter 3). Therefore, options were examined to monitor leaf N dynamics during the season with the use of optical sensors (see Chapter 6).

6 EFFICIENT USE OF THE SPAD-502 CHLOROPHYLL METER FOR MONITORING FOLIAR NITROGEN DYNAMICS OF TREE SPECIES

6.1 Introduction

Nitrogen (N) is the most used nutrient in crop production as evidenced by a substantial increase in its worldwide consumption and trade in the past decades (Craswell et al., 2004). Yet, when improperly managed, applied N is released from the soil-crop system and in turn becomes a major source of environmental concern. A judicious N management avoids not only unnecessary expenses for N fertilizers but also excessive N amendments, which may contaminate surface and groundwater bodies (Shapiro et al., 2006) or contribute to reducing emissions of N-containing greenhouse gases that often originate from N amendments (Parry, 1990). An efficient N management is thus of benefit to the land users and the environment, which has been a major motivation for the change in the N-application paradigm towards increasing N-use efficiency.

Nutrient deficiencies and toxicities are routinely diagnosed by standardized plant tissue tests based on pigment extraction and spectrophotometric determination (Munson, 1998). Undoubtedly, these are trustworthy methods, but they also necessitate considerable resources such as material, owing to the sophisticated laboratory equipment, and space, funds and time, since delays through tissue sampling and processing often prevent timely and corrective responses. The change in the paradigm of N fertilizer applications has triggered the call for cheap and easy-to-use methods and tools for real-time N management (Inada, 1963).

Given the role and functioning of N in leaves and the consequent change in leaf color in case of N deficiencies or toxicities, these spectral properties have inspired researchers to develop leaf color charts (Furuya, 1987) or optical sensors such as the SPAD (Soil Plant Analyses Development)-502 chlorophyll meter (Minolta, 1989). The latter instrument uses an *in-situ*, non-destructive means to indirectly determine total leaf chlorophyll content (SPAD indices are combined chlorophyll $a + b$ content), and thus can be repeated on the same leaves. The portable (225 g weight) meter exposes a leaf spot of 6 mm^2 to light during a short period and measures the transmission of red light with a wavelength of 650 nm, which is absorbed by the chlorophyll, and the transmission of infrared light at 940 nm, which is not absorbed. Since higher leaf chlorophyll contents absorb more light, which results in decreasing light transmittance (Havlin et al., 2005), and while assuming that the difference between the amount of light emitted by the source and that received by the sensor/detector is considered the portion of light absorbed, the captured signal is converted into an electrical signal that is displayed by the SPAD-502 as dimensionless numbers (usually between 1-99). Although the amount of light passing through the leaf is inversely proportional to the chlorophyll content, for reasons of convenience the display shows the opposite: the higher the reading, the higher the chlorophyll contents. Hence, based on the spectral reflectance ratio of the two light emitting diodes (LED), the SPAD-502 meter quantifies the leaf color. Since leaf chloroplasts contain about 70% of the leaf N (Madakadze et al., 1999), leaf chlorophyll and N contents are closely correlated (Wood et al., 1993; Richardson et al., 2002).

The SPAD-502 meter was developed in the early 1960's (Inada, 1963) particularly for improving N management in rice (*Oryza sativa* L.) (e.g. Peng et al., 1993; Singh et al., 2002). Owing to the high correlations between the SPAD-502 readings and the leaf chlorophyll and N status, the chlorophyll meter consequently has been used for numerous annual crops including maize (*Zea mays* L.) (Dwyer et al., 1994), barley (*Hordeum vulgare* L.) (Wienhold and Krupinsky, 1999), cotton (*Gossypium histutum* L.) (Wood et al., 1992), and winter wheat (*Triticum aestivum L.)* (Jifon et al., 2005). The successful use of the SPAD-502 in annual crops was an impetus for its use in woody and perennial vegetation such as apple (Neilsen et al., 1995) and cottonwood tree (*Populus deltoides* Bartr. ex Marsh.) (Moreau et al., 2004), as the necessity of N-management in tree plantations had also been recognized (e.g. Moreau et al., 2004).

In contrast, various studies show a much lower goodness-of-fit between leaf-greenness readings and leaf-N status in several horticultural crops such as strawberry (*Fragaria* x *ananassa*) (Himelrick et al., 1993) and potatoes (*Solanum tuberosum* L.) (McLaskey, 1997) and with trees such as red maple (*Acer rubrum* L.) (Silbey et al., 1996). Until now, research has not provided an answer to the question why these correlations are weaker; in perennial crops these may have been caused by leaf position and sampling time and techniques (Loh et al., 2002) or by irradiance and weather conditions given that the SPAD-502 values tended to decrease with increasing irradiance (Hoel and Solhaug, 1998). Most explanations for lower goodness-of-fit indicate, however, differences in leaf anatomy, e.g., leaf thickness. Moreover, the relationship between SPAD-502 values and chlorophyll/N content varied between simple linear and curvilinear (Dwyer et al., 1994; Moreau et al., 2004; Esfahani et al., 2008), underlining that calibration curves can be parameterized as non-linear equations (Richardson et al., 2002; Uddling et al., 2007).

Nevertheless, from the numerous findings it has been concluded that regression relationships are crop, species, cultivar and even hybrid specific (Peterson et al., 1993). This implies that outcomes cannot be transferred from one plant to another, but that site-, plant- and species-specific calibrations are necessary to determine the relationships between the SPAD-502 readings and the N content for predicting the leaf-N status (Balasubramanian et al., 2000; Richardson et al., 2002; Singh et al., 2002). Since no studies exist that have determined the relationship between SPAD-502 readings and leaf-chlorophyll and N content of tree species home to the lower reaches of the Amu Darya in Central Asian Uzbekistan, the objectives therefore were to (1) determine the feasibility of using the SPAD-502 for monitoring the leaf N content of three tree species considered for afforesting salt-affected croplands, (2) generate a calibration dataset for a rapid and inexpensive assessment of leaf N status, and (3) identify the range of the SPAD-502 readings for three local tree species that would allow prediction of N dynamics and thus support tree plantation management.

6.2 Materials and methods

6.2.1 Study sites

All measurements were conducted at two research sites in the Khorezm region of Uzbekistan: a 4-year-old tree plantation in the Yangibazar district located at 41°65′ N latitude, 60°62′ E longitude and 102 m altitude (further referred to as Experiment 1), and a 5-year-old tree plantation at the experimental site of the Urgench State University located at 41°33′ N latitude, 60°36′ E longitude and 101 m altitude (Experiment 2). For comparison and validation of the relationships between the SPAD-502 readings and tree leaf chlorophyll and N content, two factors were decisive: 1) the same tree species had to be grown on both locations and 2) the trees had to be of different ages (trees in Experiment 2 were two years older). This allowed establishing a “false time series”.

During 2008 when data was collected for the validation, the mean air temperature was approximately 17°C with minimum and maximum daily temperatures ranging from -8°C to 43°C, respectively. The annual precipitation amounted to 100 mm and fell mostly outside the tree-growing period. The mean relative air humidity varied between 26% and 86% throughout the year.

6.2.2 Experimental design

Experiment 1: In March 2006, treatments were arranged in a completely randomized block design with four replications on 0.5 ha of marginalized land. The selected tree species, namely Russian olive (*Elaeagnus angustifolia* L.), honey locust (*Gleditsia triacanthos* L.) and black locust (*Robinia pseudoacacia* L.), were subjected to two levels of phosphorus (P) application and compared to a control without P application (see Chapter 3). These fertilizer treatments included the addition of half (50%) the rate and the full rate (100%) of the recommended P rate (90 kg ha^{-1}) as single super phosphate (SSP). In the below-described analyses, the effect of different levels of P additions on the SPAD readings of tree foliage was excluded, and hence the focus is on the control treatment with no P amendments. Each experimental plot consisted of one row of 11 trees, transplanted as 1-year-old saplings from the tree nursery into the experimental plots. The trees were planted at 1-m distance in rows spaced 1.75 m, resulting in a density of 5,714 trees ha^{-1}. Given the young age of the trees, they did not interfere with each other during the entire three-year-study period from March 2006 till October 2008. Watering was ensured via drip irrigation at 80 mm per growing season, which was stopped after two years as the roots started tapping the groundwater. The soil in Experiment 1 (Table 6.1a) was classified as gleyic and calcaric Arenosols (ISEAM, 2001). During the growing seasons 2006 and 2008, the groundwater level fluctuated between 1.5 and 1.8 m.

Experiment 2: A 0.2 ha site was selected for comparison and validation of the results in Experiment 1. Trees had been planted in 2005 at the same spacing as in Experiment 1. The 13 lines consisted of 30 trees of 10 species that were used for calibration, including *E. angustifolia*, *G. triacanthos* and *R. pseudoacacia*. The individual trees were considered replicates. The soil type was the same as that in Experiment 1 (Table 6.1b). During the growing seasons, the groundwater level fluctuated between 1.4 and 1.8 m. During the first two years of tree establishment, the entire plantation was furrow irrigated, but after 2006 the trees tapped the groundwater.

Table 6.1: Physical and chemical characteristics of the soil in 2008 in Experiment 1 (a) and Experiment 2 (b)

(a)

Parameter	Soil depth (cm)		
	0-25	**25-65**	**65-110**
Bulk density (g cm^{-3})	1.2 (±0.0)	1.5 (±0.0)	1.5 (±0.0)
pH	7.6 (±0.3)	7.6 (±0.2)	7.7 (±0.2)
Dry residue (%)	0.2 (±0.1)	0.2 (±0.0)	0.1 (±0.0)
Soil organic matter (%)	1.1 (±0.0)	0.7 (±0.0)	0.6 (±0.0)
P_2O_5 (mg kg^{-1})	14.4 (±2.9)	10.3 (±3.7)	10.1 (±5.2)
K_2O (mg kg^{-1})	192 (±11)	101 (±8)	87 (±8)
Total N (%)	0.07 (±0.00)	0.05 (±0.00)	0.04 (±0.00)
Total P (%)	0.13 (±0.02)	0.15 (±0.01)	0.15 (±0.01)
Total K (%)	0.73 (±0.06)	0.77 (±0.04)	0.82 (±0.05)

(b)

Parameter	Soil depth (cm)		
	0-30	**30-60**	**60-110**
Bulk density (g cm^{-3})	1.7 (±0.0)	1.7 (±0.0)	1.7 (±0.0)
P_2O_5 (mg kg^{-1})	32.5 (±2.5)	29.1 (±2.5)	na
K_2O (mg kg^{-1})	193 (±33)	180 (±24)	na
Total N (%)	0.05 (±0.00)	0.04 (±0.00)	na
Total P (%)	0.09 (±0.01)	0.09 (±0.01)	na

6.2.3 SPAD-502 measurements

In Experiment 1, two trees per species were randomly selected within each of the four control plots for measuring the leaf chlorophyll content with the SPAD-502 meter at 14-day intervals. Three small, medium and large sized leaves (in total nine leaves per tree) were measured between the midrib and the leaf margin. The measurements started in May, when leaves began flushing, and ended in September, before natural leaf shed. The measurements were carried out for three consecutive years (2006, 2007, and 2008). Prior to each use, the SPAD-502 was calibrated following the instructions provided by the manufacturers.

6.2.4 Sampling of leaves for SPAD-502 calibration

For the elaboration of the calibration curves, leaf material was collected from three tree species. Healthy and fully expanded leaves with visible differences in leaf "greenness" were selected to maximize the calibration range database. Three SPAD-502 readings were taken between the midrib and the leaf margin of 10 leaves of each of the three species and averaged. Following these measurements, the 30 leaves with the same characteristics were picked from the trees between 8:00 and 9:00 am, the petioles were detached, and the leaves placed in polyethylene bags and transported to the laboratory in a refrigerated box. The bags were then stored in a stationary freezer at -10°C until analysis for total N using the Kjeldahl method and for chlorophyll *a* + *b* according to Lichtenthaler (1987).

6.2.5 Monitoring foliage N content with SPAD-502 and validation of results

Several functional forms were tested to identify the best goodness-of-fit and to describe the relationships between the SPAD-502 values and total chlorophyll content (mg g^{-1}), total chlorophyll and N content (mg g^{-1}), and the SPAD-502 values and total N content. When using the established equations obtained for the midseason (July 2008), based on the SPAD-502 measurements the leaf-N status could be derived at any other period. For validation of the calibration equations that were derived from regressing total N content with the SPAD-502 readings of *E. angustifolia*, *G. triacanthos* and *R. pseudoacacia* in Experiment 1, the equations were used to calculate the leaf-N content of the same tree species based on their measured SPAD-502 values at Experiment 2. Next, these simulated N values were compared with the leaf-N values of Experiment 2 as determined by spectrophotometric analyses.

6.2.6 Statistical analyses

One-way analysis of variance (ANOVA) was used to analyze differences in the SPAD-502 values for three tree species in Experiment 1 over three years. When significant ($P<0.05$) differences were found, the Tukey post-hoc test was used to compare individual means. Coefficients of determination (R^2) were computed for all tree species by regressing the SPAD-502 readings with leaf total chlorophyll content, leaf total chlorophyll with N content, and the SPAD-502 readings with leaf total N content. Statistical analyses were performed with SPSS 15.0 and SAS 9.2 software. The root mean squared error (RMSE) and relative root mean squared error (RRMSE) was used for determining the differences between the predicted N values and those determined by spectrophotometric analyses, where

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}(observed_i - simulated_i)^2}{n}} \quad (6.1)$$

$$RRMSE = \frac{RMSE}{Average(observed)} \cdot 100 \quad (6.2)$$

6.3 Results

6.3.1 Regression types and calibration equations

Although the R^2 of the linear relationship between total leaf chlorophyll and N contents and their corresponding SPAD-502 readings varied between 0.62 and 0.78 for the three species (Table 6.2a, b, c), they still had a lower predictive value than the calibration lines based on the second-degree polynomial models (Equation 6.3), which in all cases provided the highest R^2 for all relationships of interest (Table 6.2a, b, c). Hence, in all cases, the polynomial regression types were used as calibration equations, which differed among the tree species (Figure 6.1).

$$y = [a + bx + cx^2] \quad (6.3)$$

where y is total N content, a, b and c are linear and curvilinear coefficients, respectively, and x is the SPAD-502 reading.

The SPAD-502 readings showed significant goodness-of-fit with total leaf chlorophyll content for all three tree species (Figure 6.1a). The total leaf chlorophyll content of the three species was highly correlated with total leaf-N content (Figure 6.1b). SPAD-502 values are also highly and significantly correlated with the total N content in the leaves of all three species, although the R^2 (0.76) for *G. triacanthos* is 12% lower compared to the other two tree species, but still relatively high (Figure 6.1c).

Table 6.2: Functional relationships and their coefficients of determination (R^2) between the SPAD-502 readings and total leaf chlorophyll content (a), total leaf chlorophyll and nitrogen content (b), and the SPAD-502 readings and total leaf nitrogen content (c) of three tree species

a)

Species	Regression type			
	Linear	**Logarithmic**	**Exponential**	**Polynomial**
	R^2			
Elaeagnus angustifolia	0.69	0.63	0.69	0.88
Gleditsia triacanthos	0.77	0.80	0.73	0.83
Robinia pseudoacacia	0.78	0.82	0.72	0.87

b)

Species	Regression type			
	Linear	**Logarithmic**	**Exponential**	**Polynomial**
	R^2			
Elaeagnus angustifolia	0.73	0.70	0.69	0.74
Gleditsia triacanthos	0.62	0.60	0.73	0.73
Robinia pseudoacacia	0.69	0.69	0.64	0.79

c)

Species	Regression type			
	Linear	**Logarithmic**	**Exponential**	**Polynomial**
	R^2			
Elaeagnus angustifolia	0.71	0.67	0.78	0.87
Gleditsia triacanthos	0.76	0.76	0.77	0.76
Robinia pseudoacacia	0.78	0.82	0.72	0.86

Based on these correlations, the calibration equations for predicting N content were established for each tree species (Table 6.3).

Table 6.3: Second-degree, polynomial calibration models and range of the SPAD-502 readings for predicting nitrogen content and nitrogen dynamics of tree foliage; x is the SPAD-502 reading and y is total nitrogen content

Species	Calibration equation	Range SPAD-502 readings
E. angustifolia	$y = 499.23 - 14.584x + 0.1138x^2$	62-88
G. triacanthos	$y = -61.974 + 2.8801x - 0.0162x^2$	31-51
R. pseudoacacia	$y = -45.984 + 4.0641x - 0.0433x^2$	26-48

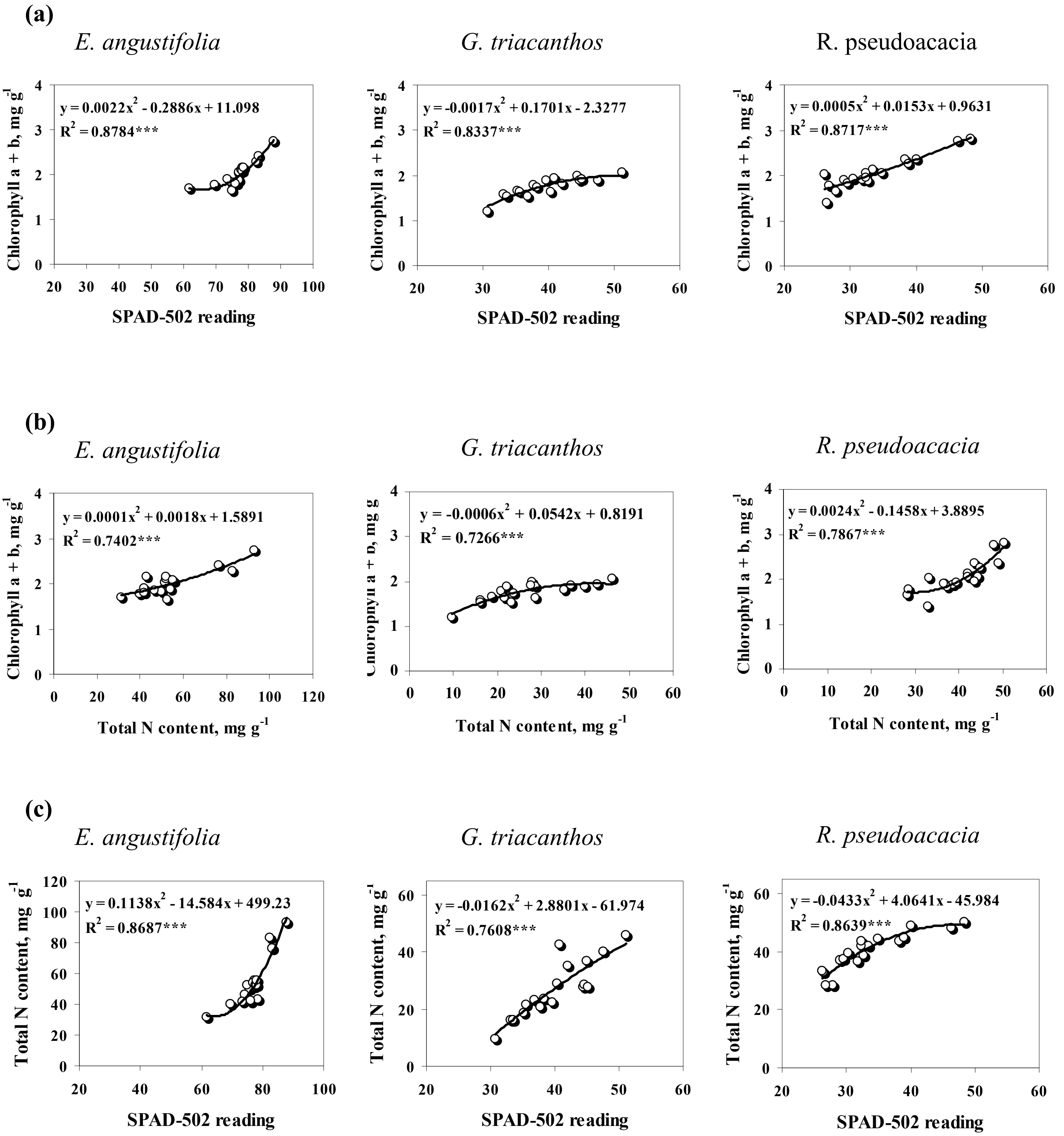

Figure 6.1: Relationships between the SPAD-502 readings and total chlorophyll content (a), between total chlorophyll and total nitrogen content (b), and between the SPAD-502 readings and total nitrogen content (c) in leaves of three tree species in Experiment 1. Statistical significance: R^2 – coefficient of determination; **P*<0.05; ***P*<0.01; ****P*<0.001

6.3.2 Validation of results

The validation of the calibration equations established for Experiment 1 show that the spectrophotometrically determined leaf N content matches very well the findings of the indirectly predicted leaf-N content irrespective of the three tree species grown at Experiment 2, as evidenced by RMSE values of 25 mg g^{-1} dry matter (DM) and 10% for *E. angustifolia*, 11 mg g^{-1} DM and 16% for *G. triacanthos*, and 17 kg g^{-1} DM and 10% for *R. pseudoacacia* (Table 6.4).

Table 6.4: Comparison of simulated and empirically (laboratory findings) determined tree-leaf nitrogen content of three species in July 2008. STDEV = standard deviation (mg g^{-1}), CV = coefficient of variation (%), RMSE = Root Mean Squared Error (mg g^{-1}), RRMSE = Relative Root Mean Squared Error (%)

Species	***Elaeagnus angustifolia***	
Parameter	**Observed mean**	**Simulated mean**
Total N content (mg g^{-1})	50	52
STDEV (mg g^{-1})	*13*	*14*
CV (%)	*26*	*27*
RMSE (mg g^{-1})	*********** *25* *********	
RRMSE (%)	********** *10* **********	
Species	***Gleditsia triacanthos***	
Total N content (mg g^{-1})	21	23
STDEV (mg g^{-1})	*4*	*4*
CV (%)	*19*	*17*
RMSE (mg g^{-1})	********** *11* *********	
RRMSE (%)	********** *16* *********	
Species	***Robinia pseudoacacia***	
Total N content (mg g^{-1})	38	41
STDEV (mg g^{-1})	*6*	*6*
CV (%)	*16*	*15*
RMSE (mg g^{-1})	********** *14* *********	
RRMSE (%)	********** *10* **********	

6.3.3 Simulating N dynamics of tree foliage for 2006, 2007 and 2007

The accuracy of the validation findings allowed the prediction of the N dynamics over the 2006, 2007 and 2008 growing seasons using the measured SPAD-502 values and calibration equations established for the three tree species at site 1 (Table 6.5). The

SPAD-502 values and the N content of the tree leaves for all tree species tended to increase over years (Table 6.5a, b).

Table 6.5: Measured SPAD-502 readings and simulated nitrogen content of leaves of three tree species in Experiment 1. ± indicates standard deviation; within a column means followed by the same letter are not significantly different at *P*<0.05 according to the Tukey post-hoc test

a)

Species	**SPAD-502 readings**		
	2006	**2007**	**2008**
E. angustifolia	60 ± 8 a	67 ± 7 a	71 ± 9 a
G. triacanthos	32 ± 3 b	32 ± 4 b	35 ± 4 b
R. pseudoacacia	29 ± 5 b	35 ± 5 b	36 ± 5 b

b)

Species	**Predicted N content, mg g^{-1}**		
	2006	**2007**	**2008**
E. angustifolia	39.07 ± 9.51 a	42.41 ± 6.28 a	52.42 ± 13.71 a
G. triacanthos	13.96 ± 3.27 b	15.99 ± 3.89 b	23.02 ± 4.13 b
R. pseudoacacia	33.91 ± 6.80 a	38.24 ± 3.82 a	41.08 ± 5.52 a

6.4 Discussion

6.4.1 Functional relationships

The efficiency and design of optical chlorophyll meters such as the SPAD-502 roots in the principle that the amount of light measured after passing through the leaf is inversely proportional to the active chlorophyll *a* + *b* content (Havlin et al., 2005). Moreover, since N is a crucial component of chlorophyll (Madakadze et al., 1999), and a highly positive relationship exists between these two parameters, the SPAD-502 can be used to determine the leaf-N content (Wood et al., 1993; Richardson et al., 2002). Despite the linear relationship between the SPAD-502 readings and the leaf-N status as has been measured in numerous studies (Balasubramanian et al., 2000; Singh et al., 2002), many other studies reported weaker linear correlations between the SPAD-502 readings and the N concentration (Loh et al., 2002). Abundant evidence shows that the functional relationship between the SPAD-502 values and chemical leaf parameters over a range of crops appears to differ according to species, cultivars, and even crop growth stage (e.g. Takebe and Yoneyama, 1989; Turner and Jund, 1991; Peterson et al., 1993). In fact, any biotic (e.g., pests, diseases, weeds, etc.) or abiotic stress (e.g., drought, heat, cold, salinity) that impacts leaf discoloration will impact the SPAD-502 values and chlorophyll estimates (e.g. Turner and Jund, 1991; Peterson et al., 1993; Balasubramanian et al., 2000). Furthermore, the type of relationships between the SPAD-502 readings and leaf-N and chlorophyll content may also differ (Castelli et al., 1996; Richardson et al., 2002). All these findings indicate that functional relationships must be developed for each of these parameters to increase the efficiency of the SPAD-502 meter.

In contrast with various previous studies, the best goodness-of-fit of the functional relationship identified was not linear but curvilinear. In some cases it is desirable that the model or functional relationship is as simple as possible, which would call for a linear relationship. Yet, when aiming to use the model results for predicting the leaf-N content based on corresponding, non-calibrated SPAD-502 values, it is highly recommended to use the relationship with the highest coefficient of determination (Kumar, 2008), which in this study was the second-degree polynomial relationship for all three tree species.

6.4.2 Range of SPAD-502 readings

Obviously, predictions are only valid within the SPAD-502 range of readings identified during the calibration process, which differed among the tree species (Table 6.4). Nonetheless, based on an intensive monitoring of all three species during three consecutive seasons, and given the accuracy of the outcomes, the possible range of the SPAD-502 values for each of the species has been covered, which in turn allows using the established curvilinear relationships (Table 6.4). This outcome is also supported by a comparison of the leaf-N contents for the three tree species as determined by spectrophotometric analyses and with the established relationships for the same species (Table 6.3). Hence, the SPAD-502 meter can now be used for leaf-N determination and prediction for the three woody species examined in the study region.

Despite the high goodness-of-fit, the coefficients of determination differed among the surveyed tree species (Tables 6.2a, b, c). Various reasons have been suggested for such differences, including the nature of the sampling technique, since the leaf spots used for the SPAD-502 readings do not always correspond truthfully to that leaf part used for the N determination by spectrophotometric analyses, or differences in leaf thickness (Loh et al., 2002). Even weather conditions and radiation differences that occur over the growing season or during the day reportedly may impact the goodness-of-fit of the relationship (Hoel and Solhaug, 1998). However, diurnal fluctuations in the SPAD-502 readings (data not shown) could not be detected during the hot summers in the study region. Furthermore, by averaging three SPAD-502 readings for each leaf to one value, a potential sampling technique error was reduced. Regarding the leaf thickness, which can be quantified by the specific leaf area (SLA: the greater the SLA, the thinner the leaf), previous research findings on various tree species in the study region showed indeed significant differences between species with respect to this trait (Lamers et al., 2006). From this perspective it can be argued that the coefficients of determination should be more elevated for tree leaves with a large SLA, e.g., leaves of *E. angustifolia* (SLA = 0.031 m^2 g^{-1}), and lower for species with potentially thicker leaves such as *F. pennsylvanica* (SLA = 0.014 m^2 g^{-1}) and *U. pumila* (SLA = 0.009 m^2 g^{-1}) as determined in a previous study (Lamers et al., 2006). Although this confirms the initial line of argumentation, the same study showed that the SLA of the same species differed between years and, for instance, decreased for some species, increased with others and remained unchanged for another group of species over the course of 12 months. This suggests that the SLA may in the long run not be the most judicious trait to examine. Even though SLA or specific leaf weight (SLW; inverse of SLA) may seem promising for adjustments (Loh et al., 2002), one of the key advantages of the SPAD-502 as a non-destructive means for determining leaf-N content gets lost compared to non-adjusted SPAD-502 readings because the determination of the SLA or SLW will again demand destructive procedures.

6.4.3 Validation of results

The leaf-N values of *R. pseudoacacia* were close to those reported by Singh (1982) (>3%), whereas for *G. triacanthos* the values provided by Perry and Hickman (1999-2000) match the present findings very well (2.3%). The N content in *E. angustifolia* leaves as determined during an afforestation study in the same region was about 3-4% (Khamzina et al., 2006), which is slightly lower than the values in this study. The leaf-N content and consequently the SPAD-502 readings of this species were much higher than those of the other species, which can be explained by the species' effective N_2-fixing capacity, which has also been reported in earlier studies (Khamzina et al., 2009a). The results are similar for the predicted leaf-N content (Table 6.5b). However, based on the low RSME values (Table 6.3), the relationships established for *E. angustifolia*, *G. triacanthos* and *R. pseudoacacia* turned out to be sufficiently accurate and thus can be used.

The confirmation that the SPAD-502 meter can be used for N determination and prediction for three species has practical implications for tree and forest management in the lower reaches of the River Amu Darya. For instance, given the growing interest in woody vegetation for fiber and bio-energy production on marginal land as one avenue to avoid the dilemma of biomass use for food or fuel production as well as for carbon sequestration, there is a definite need for managing tree productivity via appropriate N-management (Moreau et al., 2004). This seems critical also within the irrigated areas in the lower reaches of the Amu Darya in Uzbekistan (Martius et al., 2004). Recent findings have shown the potential role of small-scale tree plantations as a means to re-convert degraded marginal lands into areas suitable for multipurpose tree plantations (Khamzina et al., 2006b; Lamers et al., 2006; Khamzina et al., 2008; Lamers et al., 2008). In addition, the global warming potential of N_2O and CH_4 fluxes from annual cropping systems such as cotton, wheat and rice, were shown to be much higher than from perennial systems such as the *tugai* and poplar tree plantations (Scheer et al., 2008). However, for the bio-remediation of these degraded, marginal lands, tree species need to be assorted (Khamzina et al., 2006) and once planted, adequately managed. Here, the SPAD-502 readings can be used for the species examined in this study.

6.4.4 Contribution of SPAD-502 to N management

Furthermore, recent studies have underlined the importance of animal husbandry for livelihood sustainability in rural Uzbekistan (e.g. Müller, 2006; Kan et al., 2008). Yet, the notorious shortage in feed supply is a major cause for the poor livestock productivity in the region (Djanibekov, 2006), and feed diets need to be improved. Referring to a foliar crude protein (CP) content between 90 and 150 g CP kg^{-1}, and a nutritive value in tree leaves, which relative to barley (100) varied from 62 (*P. euphratica* L.*)* to 97 (*E. angustifolia* L.) (Lamers and Khamzina, 2010), it was postulated that tree leaves could be used to complement and improve the presently low-quality diet of livestock. Based on the assumption that the N content of CP as one of the major feed quality indicators is 16% (Close and Menke, 1986), the SPAD-502 can be used to monitor not only leaf-N but also leaf-CP content during the growing season (CP=N·6.25). This would consequently support livestock holders in their choice for the best time to use tree leaves and thus improve and enrich the quality of poor fodder.

Last but not least, although the leaf greenness as indicated by the SPAD-502 readings can become a suitable predictor of plant growth and thus can provide directives for the management of N amendments, the SPAD-502 meter is a diagnostic rather than a prognostic tool. The arbitrary indices indicate whether or not plant leaves are sufficient in N, but do not reveal how much N to apply unless threshold values are established such as for various rice cultivars (Singh et al., 2002). Under such conditions, the SPAD-502 can contribute directly to an increased N use efficiency and biomass production of crops and trees.

6.5 Conclusions

The reliable correlations between the SPAD-502 readings and the leaf chlorophyll and N status for three tree species as well as the validation of the results for these species indicate that the established curvilinear relationship can be used for rapid, non-destructive and indirect but accurate estimations of the leaf-N status as long as the SPAD-502 values are within the established calibration database. This in turn has various practical implications for tree management and could support, for instance, livestock holders in the lower reaches of the Amu Darya in Uzbekistan in selecting the appropriate species, leaves and time of leaf grazing for improving and enriching feed diets (see Chapter 7).

7 OPTIONS FOR OPTIMIZING DAIRY FEED RATIONS WITH FOLIAGE OF TREES GROWN IN THE IRRIGATED DRYLANDS

7.1 Introduction

Ample evidence underlines the benefits of supplementing ruminant diets with tree foliage to complement feed particularly low in nitrogen (N) and crude protein (CP) contents (Devendra, 1992; Humphreys, 1995; Reddy and Elanchezhian, 2008). Such practices however have not been introduced in the dryland areas of Central Asia (CA) owing to an unawareness of, and knowledge about such benefits of perennial vegetation. Yet, following independence from the former Soviet Union (SU) in 1991, livestock has grown tremendously in importance and has become vital for livelihood security of the rural population of the five CA countries, Kazakhstan, Kyrgyzstan, Tajikistan, Turkmenistan, and Uzbekistan (Iñiguez et al., 2005). In the aftermath of independence, a drastic decline in the number of livestock has occurred, although the present stock is again estimated at 17 million (mi) cattle, 44 mi. sheep, and 7 mi. goats (Iñiguez et al., 2005).

Although animal husbandry has become central to the livelihood in rural Uzbekistan (Müller, 2006; Kan et al., 2008), feedstuff demand is outstripping its production. Animal keepers in the Khorezm region of Uzbekistan for instance mix CP-deficit and relatively rich metabolizable energy (ME) feedstuff such as wheat bran (CP:ME=9.7 g MJ^{-1}), or straw of maize (CP:ME=9.4 g MJ^{-1}) or sorghum (CP:ME=6.6 g MJ^{-1}) with CP-rich feeds such as cottonseed cake (CP:ME=29.9 g MJ^{-1}), when affordable and available (Djanibekov, 2008). But in the absence of the expensive cottonseed cake, the notoriously low quality of the cheap grain bran and fibrous roughages is one cause for the poor livestock productivity of, e.g., 6-7 litres per day per cow (Djanibekov, 2008).

A screening of potentially suitable multipurpose tree species for afforesting degraded marginal lands in the Khorezm region demonstrated the benefits of various non-timber products including the nutritive value of the tree foliage of various species (Lamers and Khamzina, 2010). At the end of the growing season the tree foliage of different species ranged within 90-150 g CP kg per dry matter (DM), but during the season CP values of 250 g CP kg^{-1} DM had been monitored and hence it was concluded that the foliage of these tree species could supplement the N–deficit diet for dairy cattle (Lamers and Khamzina, 2010). The tree leaves could thus increase the quality of the feed whereas the usual fibrous roughages (wheat, rice and sorghum residues) could complement the quantity of the feed particularly during feed deficit periods (November-May).

Although little is known about the role of the foliage from woody species grown in the irrigated croplands of CA, there is much evidence on its importance in complementing feed diets worldwide (e.g. Baumer, 1992; El-Waziry, 2007). But most studies have focused on analyzing the use of tree foliage *per se* or compared the suitability of different tree species (e.g. Rubanza et al., 2005; Amanullah et al., 2006; Al-Soqeer, 2008), analyzed the chemical structure of tree foliage (Amanullah et al., 2006; El-Waziry, 2007; Al-Soqeer, 2008) or looked at the benefits of tree foliage for different types of livestock (Baumer, 1992; Azim et al., 2002). Yet, information about the optimal timing for collecting tree leaves as to complement dairy diets, whilst considering both nutritive and financial aspects, has received little attention particularly in the irrigated drylands of CA. This demands knowledge about the seasonal dynamics of N and CP contents in tree and bush foliage. Labri et al. (2008), for instance, summarized the nutritive value of seven perennial species native to Uzbekistan, but the foliage material was only collected once during the season. The analyses by Lamers and Khamzina (2010) on the dynamics of the nutritive value of tree foliage during the eight-month growing season was based on measurements taken only three times. But even though the quality profile and the dynamics of the chemical composition of the tree foliage in this case was monitored throughout the season (e.g., Lamers and Khamzina, 2010), the methods used for the determination of the N and CP contents of the tree leaves were expensive and time-consuming. The latter hampers that the laboratory results become rapidly and timely available. This leaves the animal holders uncertain about the best time to feed tree leaves. A rapid, easy-to-use, cheap and practical method to monitor the tree leaf N/CP contents throughout the growing season would therefore allow to determine the best timing from both a nutritive and financial perspective.

The SPAD (Soil Plant Analyses Development)-502 chlorophyll meter (Minolta, 1989) compels a non-destructive means to determine total leaf chlorophyll, which is highly correlated with the leaf N content (Girma et al., 2006; Pinkard et al., 2006). However, since this relationship depends on crops, species, cultivars and even hybrids (Peterson et al., 1993), the relationships between the SPAD readings and the N/CP content has to be determined first, in particular since they vary between simple linear to curvilinear for annual and perennial vegetation (Dwyer et al., 1994; Moreau et al., 2004; Esfahani et al., 2008).

This component focuses on the introduction of tree foliage into farm management practices to improve nutrient composition of the dairy feed rations and sustain farm income. A mathematical programming model can be a tool, in this case, to formulate an optimal ration for feeding dairy cows (Holler et al., 1969). A linear programming (LP) approach, which is based on mathematical programming, has been used extensively in farm management to formulate least-cost dairy rations (O'Connor et al., 1989; Tozer, 2000; Van Calker et al., 2004). The application of LP models allows evaluating if the introduction of tree foliage into the dairy ration is both economically and technically feasible given the nutritive requirements of the diet of dairy cows, the forage nutrient composition and regional prices of feeds available in the study region Khorezm.

The objectives were therefore threefold: (i) to analyze the suitability of the SPAD-502 meter for monitoring the N and CP content in the leaves of the fodder species *Elaeagnus angustifolia* L., *Gleditsia triacanthos* L. and *Robinia pseudoacacia* L., (ii) to detect thus the best harvesting time and the effect of including CP-rich tree leaves into feed rations of dairy cows and (iii) if so, what admixture would bring about the optimal feeding ration for dairy cows at the lowest cost using the LP approach.

7.2 Description of the study region

Large parts of CA belong to the non-tropical drylands with a continental climate, characterized by short, cold and moist winter periods lasting between November and March, and long, hot and dry summers spanning from April till October. Typical for this dryland area of 392,670 million ha (Mha) are the smaller areas of irrigated landscapes amounting to less than 3% (about 8-11 Mha), but which are vital for the welfare and livelihood of the population (PFU, 2008).

With a size of 605.000 ha, of which only about 270.000 ha can be irrigated, the study region Khorezm is typical for the irrigated lowlands within CA (PFU, 2008). The Khorezm region is located between 60.05 and 61.39 N and 41.13 and 42.02 E of the Greenwich meridian, and thus, at about 250 km south from the present Aral Sea shores. The region has borders with the Karakum and Kyzylkum deserts, the Amu Darya River, the Autonomous Republic of Karakalpakstan, and Turkmenistan. The mean annual air temperature is 13°C, but daily maxima can be as high as +45°C and minima as low as -28°C. The long-term mean annual precipitation of about 100 mm, which falls mostly outside the growing season, is much lower than the annual evaporation of 1200 mm, which makes agriculture possible with irrigation only. The agro-climatic conditions are suitable for the cultivation of crops adapted to higher summer temperatures such as cotton, tobacco, and sunflower but also maize and sorghum. More details are presented in section 2.2.

Based on a *bonitet* of <40, (the *bonitet* is "the comparative assessment of the land quality and productivity with a representative level of agricultural activity" (FAO, 2003), which is rated on a 100-point scale (Ramazanov and Yusupbekov, 2003) about 20% of the irrigated area (about 37,000 ha) of the Khorezm region has become unsuitable for cropping mainly owing to soil degradation caused by ill-managed irrigation and production practices (Martius et al., 2004).

7.3 Materials and methods

In March 2006, a three year tree experiment was set up as a randomized complete block design with four replications on a 0.5 ha marginal site in the Yangibazar research station (41°65′ N latitude, 60°62′ E longitude, altitude 102 m asl) in the Khorezm region. Next to the tree species *E. angustifolia*, *G. triacanthos* and *R. pseudoacacia*, the treatments included three levels of phosphorus application. These fertilizer treatments will not be discussed here but are presented in Chapter 3. Each experimental plot consisted of one row of eleven trees, transplanted as 1-year-old saplings from the tree nursery into the experimental plots at 1 m distance inside the row and 1.75 m between the rows. Given the young age of the trees, they did not interfere with each other during the entire three-year study period. Watering was ensured via drip irrigation at 80 mm per year, which was ceased after two years as the roots started tapping the groundwater. The emitters were installed at about 10 cm from the tree basis. During the growing season the groundwater fluctuated between – 1.2 and 1.6 m in 2006, 1.5 and 1.7 m in 2007, and 1.5 – 1.8 m in 2008. Soil texture at the research site was previously identified as Salids

(USDA, 1996), or salic Fluvisols (FAO, 2006b), despite the absence of periodical waterlogging.

7.3.1 Feed quality estimates of tree foliage

Since detailed information on the quality of feedstuff in Uzbekistan included the qualitative indicators ME and CP, crude lipids (CL), crude fibers (CF), crude ash (CA), and N-free extractives (NFE), for the sake of comparison these indicators were required also for the tree leaves. Gas production (Gb), used to estimate the ME content, was measured in the presence and absence of polyethylengglycol (PEG), following the *in vitro* Hohenheim gas technique (Menke et al., 1979) . The rumen fluid was taken from a sheep (*Ovis aries*) as the donor animal that were fed 600 g grass hay and 600 g mixed concentrates, divided into two equal meals a day. The difference between the Gb with and without PEG indicates the presence of tannins (Makkar, 1993). Leaf N content was measured according to Kjeldahl, whilst the CP content was computed as the leaf N content times 6.25 (Close and Menke, 1986). Leaf ME contents were computed using the multi-regression equation developed for roughages, which considers Gb, as well as CP, and CL contents (Menke et al., 1979). The *in vitro* organic matter digestibility (dO) was subsequently derived from the multiple regression of dO on Gb, CP and CA (Menke et al., 1979).

7.3.2 SPAD-502 measurements and calibration

Two trees per species were designated within each plot for measuring fortnightly the leaf chlorophyll content with a SPAD-502 meter. Three small, medium and large sized leaves (in total nine leaves per tree) were measured between the midrib and the leaf margin. The measurements started in April when leaves began flushing and ended in October before the complete harvest of these trees.

For the calibration of the SPAD-502 values and the N/CP contents, leaf material was collected in the middle of the season (July) from three trees per species. First the SPAD-502 readings were taken between the midrib and the leaf margin of 18 leaves of each the three species and repeated three times to obtain a leaf average. Following these measurements, the same 54 leaves were handpicked from the trees between 8:00-9:00 am, petioles were detached, and leaves were placed in polyethylene bags and transported to the laboratory within a refrigerated box. In the laboratory the bags with fresh leaf samples were kept in a stationary freezer at -10C° until analysis for total N using the Kjeldahl method and for chlorophyll *a* and *b* according to Lichtenthaler (1987).

7.3.3 Dry matter production of leaves

Three trees of each tree species were completely defoliated at the beginning (May), middle (July) and end (September) of the season for leaf dry matter determination. Immediately after harvest, leaf sub-samples were weighed with a digital scale to the nearest mg, wetted and transported in a refrigerated box to the laboratory. The sub-samples were then placed into paper bags and oven-dried at 103º ± 2 0C for 72 hours to constant weight in a forced air convection oven (MacDicken et al., 1991).

7.3.4 Monitoring crude protein contents with SPAD-502

The relationship between the SPAD-502 chlorophyll meter readings and the leaf chlorophyll and N contents were analyzed. Several functional forms were tested, but the curvelinear relationship gave the highest coefficient of determination (R^2) for all three species (see section 6.3.1). The general equation can be described as $y = a + bx + cx2$, where y is total nitrogen content, a, b and c are linear and curvilinear coefficients respectively, and x is the SPAD-502 reading. While using the established coefficients obtained for the midseason (July), the leaf N status could be derived at any other period following SPAD-502 measurements. The leaf CP contents were computed as aforementioned (Close and Menke, 1986). Here we report the CP contents at the season-onset (May), the mid-season (July) and end of the growing season (September). The accuracy of the regressed CP values was validated with CP values of the same species and in the same month but as measured in the laboratory. The accuracy is reflected in the coefficient of variation (CV), root mean squared error (RMSE) and the relative RMSE (RRMSE). For more details see section 6.2.6.

7.3.5 Feed quality estimates of feedstuff

The leaf dO values for the tree species were estimated *in vitro*. However, information on the dOs of the commonly used feedstuff in the study region such as hull, wheat and rice straw, wheat bran, maize stems, alfalfa, and maize and sorghum straw as well as cottonseed cake had not been previously reported (Dalakyan et al., 1980). Therefore, dO values of the above mentioned feedstuff was derived indirectly from available information in a step-wise procedure. First, the Gb for each feed was calculated using the multiple regression equation for roughages with the square of CL (Menke et al., 1979). In a second step, the values for the estimated Gb, and the measured CP and CA values were used for calculating the dO, according to the multiple regression equation as previously derived by Menke et al. (1979).

7.3.6 Statistical analyses

One-way analysis of variance (ANOVA) was used to analyze differences in leaf dry matter (DM) production as well as CP content of tree species at the three examined periods of the growing season. When significant ($P<0.05$) differences were found, the Tukey post-hoc test was used to compare individual means. Statistical analyses were performed with SPSS 15.0.

7.3.7 Optimization procedures

To analyze the optimization of a feed mix based on the various feeds, a linear programming (LP) model was elaborated, including the commonly used livestock feed in the study region (Djanibekov, 2006). The nutritive values of these feeds were taken from Dalakyan et al. (1980). Key elements of the LP are the feed prices, the CP and ME components of these feeds and the nutritional requirements to obtain a predefined level of daily milk production based on the body mass of a cow. Under the assumption that dairy producers would use feed at a minimum costs for the dairy feed ration while striving for a suitable CP:ME ratio, the objective function of the least cost ration (LCFR) model was:

Minimize $Z = C'X$

Subject to $AX = or \geq B$

and $X > 0$

where

Z = cost of 1 kg DM of feed mix;

C = price of 1 kg DM of feed;

A = nutrient attributes of 1 kg DM of modeled feeds in CP, ME, CA, CL, CF, NFE and Gb;

B = required nutritional constraints;

X = share of feeds in 1 kg DM of feed ration introduced as positive variables.

Therefore each feedstuff was defined by its attributes (A) relative to the feed quality indicators CP (g CP kg^{-1} DM) and ME (MJ kg^{-1} DM) contents. The final, optimal LCFR should meet the required nutritional constraints (B). Therefore, the model first sums the values of the decision variables to 1000 g, on which basis the least expensive feed mix is composed. Concurrently, the maximum and minimum DM was considered for different levels of milk production and body mass of a lactating cow (Close and Menke, 1986). These levels were imposed for fresh weights and dry weights of the fodders modeled.

Subject to the model assumptions, a linear relationship between ME requirements and milk production was established with the constant structure of the feed ration as long as the maximum fresh and dry weight constraints are not violated. According to previous research (Close and Menke, 1986) various constraints were imposed to obtain a well-balanced diet for milk production: (i) a CP:ME ratio of at least 13 g MJ^{-1} and (ii) the dO of the mix should be not less than 70%. The model was formulated and solved via the MS Excel Solver. According to the LP model, the costs of one kg DM of the feeding ration is minimized as defined by market prices of fodders (C) in USD kg^{-1} while changing the feed composition in fodders (X). The LP model estimated the least cost combination of various ingredients in a 1 kg DM feeding ration that would meet the nutrient requirements for various levels of daily milk yield and body mass of a cow. During these estimations it was assumed that a lactating cow with a weight of 400 kg produced 6 kg of 3.5% fat milk per day with a zero daily weight gain, which is considered average in the study region. This would require a daily diet of 74.7 MJ of ME and 845.2 g of CP. In the financial analysis, a period of 300 lactating days was assumed, which is the standard lactation period in the study region.

Since the nutritional value of fodder leaves and their quantity varied over the growing season (Table 7.1), the model considered these empirically observed dynamics of nutritional quality and quantity of tree leaves. This allowed identifying both the most suitable tree species and the best time of the growing season (here month) for harvesting leaves for dairy feed. The periods examined included May, July and September corresponding to the selected field measurements by the SPAD-502. It was assumed also that the nutritional quality of crops and crop by-products was constant over time in the absence of more detailed information.

Table 7.1: Dry matter (DM) production and crude protein content (CP) of three tree species over three periods of the growing season 2007. DM and CP values within one column followed by the same letter are not significantly different at $P<0.05$ according to the Tukey post-hoc test; values in brackets are standard deviations of the means

Species	***Elaeagnus angustifolia***		
Months	**May**	**July**	**Sept**
Parameter			
DM (kg ha^{-1})	567 (±137) a	1696 (±329) a	2223 (±534) a
CP (g kg^{-1})	250 (±13) a	201(±19) a	227(±11) a
Species	***Gleditsia triacanthos***		
DM (kg ha^{-1})	25 (±2) b	32(±7) b	87 (±34) b
CP (g kg^{-1})	129 (±6) b	114 (±8) b	109 (±6) c
Species	***Robinia pseudoacacia***		
DM (kg ha^{-1})	118 (±24) b	487 (±179) b	804 (±64) b
CP (g kg^{-1})	221 (±19) b	211 (±17) a	201 (±5) b

7.3.8 Feed and milk prices

Feed and milk prices were obtained from fodder and livestock market surveys in Urgench city of Khorezm in August 2008. They were collected in the local currency Uzbek Soum (UZS kg^{-1}) but converted to USD using the exchange rate of 1,319 UZS USD^{-1}. Since tree leaves were not sold at the regional markets, shadow prices were derived via regression analysis using the information of the May market prices, CP:ME content and dry matter (DM) content of nine locally marketed feeds. Next, using the information on the seasonal dynamics of leaf DM production (in kg ha^{-1}), the prices of tree leaves in July and September were calculated under assumption that the total value of leaf DM production (in USD ha^{-1}) was constant.

7.3.9 Sensitivity analyses

To determine the LCFR with and without the inclusion of tree leaves and the best period of using tree leaves based on the nutritive values, four scenarios were simulated. In the baseline scenario 1, an optimal feed mix was estimated while excluding tree leaves all together. This simulation thus represented an LCFR mirroring the current conditions of livestock holders in the study region. The resulting LCFR served as the benchmark for comparison when allowing feed admixture with tree leaves in the subsequent three scenarios. In scenario 2, tree leaves with a feed quality as monitored in May were included in the feed mix, without altering the other parameters. Scenario 3 included only tree leaves with a feed quality for July, and in scenario 4, only foliage with the nutritional values as determined in September.

7.4 Results

7.4.1 Calibration of SPAD-502 for three tree species

Although in all cases a higher SPAD-502 reading corresponded to higher N (and CP) content, the relationship between these parameters differed between the species. Whereas the coefficient of determination (R^2) of *G. triacanthos* did not vary between the linear and curvilinear regressions, the R^2 for the other two species was highest in the case of a curvilinear relationship with a polynomial regression type (Table 7.2).

Table 7.2: Coefficients of determination (R^2) for linear and curvilinear relationships derived from the regressed SPAD-502 readings to the leaf N content (mg g^{-1}) of three tree species. Same as table 6.2c

Species	**Regression type**			
	Linear	**Logarithmic**	**Exponential**	**Polynomial**
	R^2			
Elaeagnus angustifolia	0.71	0.67	0.78	0.87
Gleditsia triacanthos	0.76	0.76	0.77	0.76
Robinia pseudoacacia	0.78	0.82	0.72	0.86

The high R^2 between N content and the SPAD-502 readings for all three species provided confidence that its leaf N, and consequently CP, content could be predicted at all times (Figure 7.1). The SPAD-502 readings were highest for *E. angustifolia* ranging in July between 62 and 88, whereas for the other two species ranged between 27 and 51 over the same period. This confirmed that the period of July was the optimal period for establishing the calibration curves. Consequently, the N content of the randomly selected leaves varied in *E. angustifolia* from 32 to 90 g N kg^{-1} DM and in the other two species from 10 to 50 g N kg^{-1} DM, thus indicating higher CP contents in *E. angustifolia* leaves.

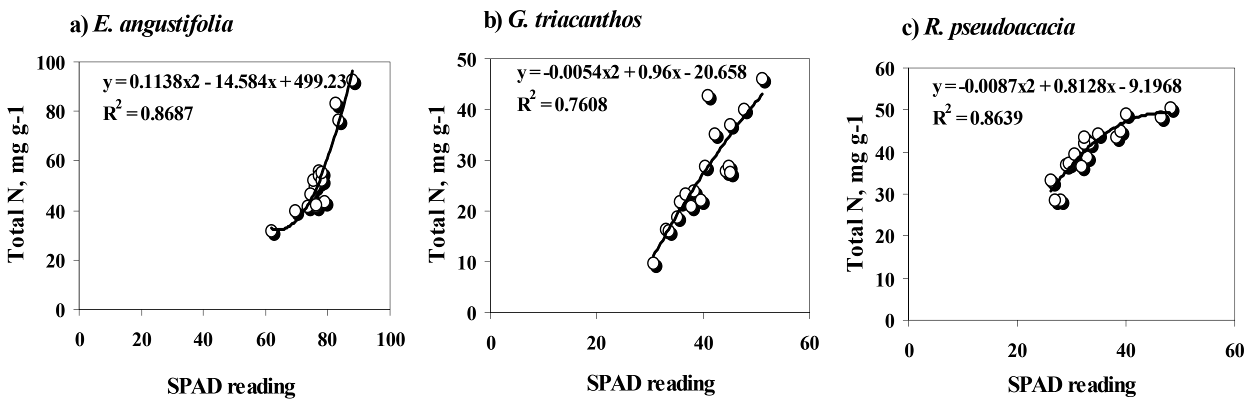

Figure 7.1: The relationship between the SPAD-502 readings and total N content of three tree species: *Elaeagnus angustifolia* (a), *Gleditsia triacanthos* (b) and *Robinia pseudoacacia* (c). Same as figure 6.1c

7.4.2 Accuracy of crude protein content estimation

The directly measured (laboratory findings) CP contents matched well the findings of the indirectly predicted leaf CP contents (based on the SPAD-502 readings) irrespective of the tree species as evidenced by the low CVs, and an RMSE of 25 g kg^{-1} DM and a RRMSE of 10% for *E. angustifolia*, 23 g kg^{-1} DM and 18% for *G. triacanthos* and 20 g kg^{-1} DM and 9% for *R. pseudoacacia* (Table 7.3).

7.4.3 Evaluation of feeds

Compared to the CP:ME ratio of ~13 g MJ^{-1} of feedstuff, which is considered a ratio with a high potential for dairy cattle (Close and Menke, 1986), the common feeds available in Khorezm were of low quality (Figure 7.2) due to their low CP and relatively high ME contents. Exceptions are alfalfa and cottonseed cake. In contrast, the high CP:ME ratio of the tree leaves, resulting from (very) high contents of CP but relatively low ME values, demonstrated the potential of these leaves as a suitable supplement to feedstuff containing low CP and relatively higher ME contents. This mix would give a suitable CP:ME ratio and thus potentially enrich the present dairy feeds.

Table 7.3: Comparison of modeled and empirical crude protein (CP) contents of three tree species in the beginning (May), middle (July) and end (Sept) of the 2007 growing season. STDEV = standard deviation (g kg^{-1}), CV = coefficient of variation (%), RMSE = Root Mean Squared Error (g kg^{-1}), RRMSE = Relative Root Mean Squared Error (%).

Species			***Elaeagnus angustifolia***			
Months	**May**	**July**	**Sept**	**May**	**July**	**Sept**
Parameter	**Based on SPAD-502**			**Based on analyses**		
CP (g kg^{-1})	250	201	227	236	182	190
STDEV (g kg^{-1})	*13*	*19*	*11*	*20*	*29*	*25*
CV (%)	*5*	*9*	*5*	*8*	*16*	*13*
RMSE (g kg^{-1})			*********** *25* *********			
RRMSE (%)			********** *10* **********			
Species			***Gleditsia triacanthos***			
CP (g kg^{-1})	129	114	109	168	123	103
STDEV (g kg^{-1})	*6*	*8*	*6*	*47*	*22*	*26*
CV (%)	*5*	*7*	*6*	*28*	*18*	*25*
RMSE (g kg^{-1})			********** *23* *********			
RRMSE (%)			********** *18* *********			
Species			***Robinia pseudoacacia***			
CP (g kg^{-1})	221	211	201	214	186	178
STDEV (g kg^{-1})	*19*	*17*	*5*	*33*	*18*	*15*
CV (%)	*9*	*8*	*2*	*15*	*10*	*8*
RMSE (g kg^{-1})			********** *20* *********			
RRMSE (%)			********** *9* **********			

7.4.4 Optimization of feed rations

The LCFR simulations (Table 7.4) were very sensitive to the introduction of tree leaves into the dairy feed ration as it allowed achieving a CP:ME ratio of ~13 g MJ^{-1} while decreasing at the same time the cost of the feeding ration. The introduction of tree leaves into the feed mix substantially reduced the need for expensive cottonseed cake whereas low quality maize stover could be completely removed from the feeding ration (Table 7.4). In contrast, the share of grain bran in the feed mix increased when tree leaves were introduced. The cheapest feed was a mix of cottonseed husk and wheat bran complemented with leaves of *G. triacanthos* in May and with *E. angustifolia* leaves in July.

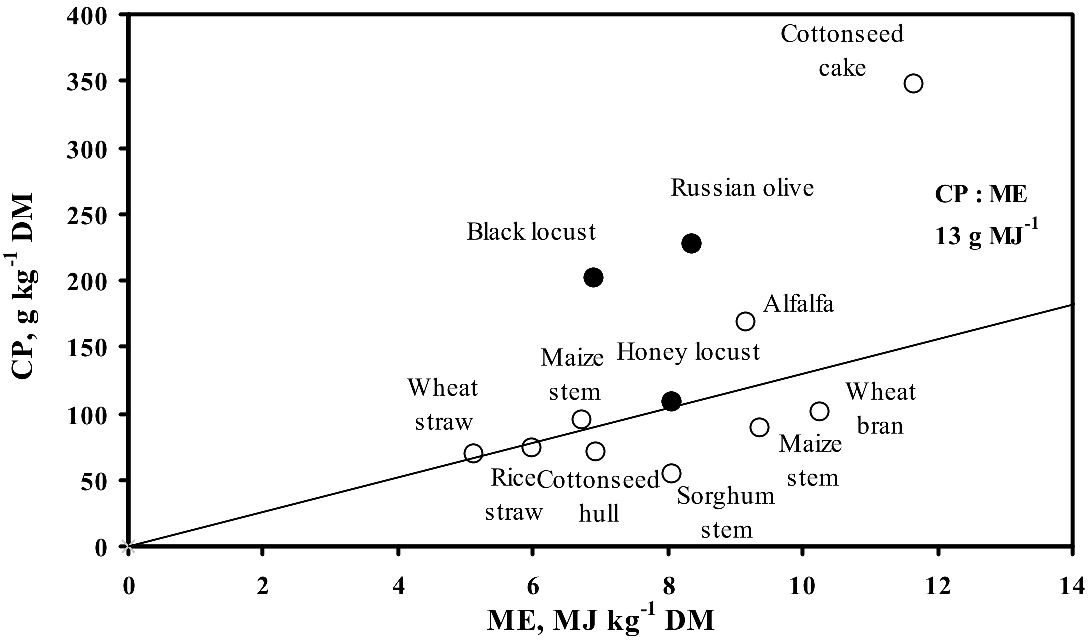

Figure 7.2: Crude protein (CP) and metabolizable energy (ME) contents of commonly used feedstuff in Khorezm (open dots) compared to the values of the examined tree leaves (black dots) and as compared to the CP:ME ratio of 13 g MJ^{-1}

Inclusion of the tree leaves into the feed mix hardly changed the dry weight of the ration or the ME content although the fresh weight of the diet decreased considerably. Consequently, while the cost per kg of dry matter of the dairy feed ration decreased by less than 10% when including the tree leaves, the cost per kg fresh weight of the diet decreased in May by 37%, in July by 22%, and in September by 19%. The findings or the LCFR model simulations showed that mixing tree leaves with the common feedstuff would increase profits in May by 53%, in July by 38% and in September by 34% (Table 7.4).

Table 7.4 LCFR Model specification andsimulation

Objective function value, USD kg^{-1}DM Run 1	Run 2	Run 3	Run 4
0.276	0.245	0.253	0.256

Activities	Unit	RHS	Run 1	Run 2	Run 3	Run 4	X1 Cottonseed cake	X2 Cottonseed hull	X3 Wheat bran	X4 Wheat straw	X5 Rice straw	X6 Maize stem	X7 Maize, whole plant	X8 Alfalfa	X9 Sorghum, whole plant	X10a Elaeagnus angustifolia (May)	X11a Robinia pseudoacacia (May)	X12a Gliditsia triacanthos (May)	X10b Elaeagnus angustifolia (July)	X11b Robinia pseudoacacia (July)	X12b Gliditsia triacanthos (July)	X10c Elaeagnus angustifolia (September)	X11c Robinia pseudoacacia (September)	X12c Gliditsia triacanthos (September)
Price, USD kg^{-1} DM							0.47	0.30	0.27	0.15	0.15	0.10	0.30	0.46	0.29	0.17	0.11	0.13	0.06	0.03	0.10	0.04	0.02	0.03
Activity levels, % in mix DM Run 1							0.13		0.78			0.10				-	-	-	-	-	-	-	-	-
Run 2							0.09		0.60									0.31	-	-	-	-	-	-
Run 3							0.07		0.79							-	-	-	0.13			-	-	-
Run 4							0.07		0.82							-	-	-	-	-	-	0.11		
Constraints																								
DM Weight	gr DM	1,000 =	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
CP : ME	g MJ^{-1}	13.0 =	13.0	13.0	13.0	13.0	29.9	10.2	9.7	13.4	12.4	14.2	9.4	18.3	6.6	28.2	34.5	13.5	23.2	35.6	12.3	27.1	29.0	13.5
Digestibility of organic matter (dO)	% DM	70.0 ≤	70.0	70.0	70.0	70.0	79.0	48.4	70.6	42.4	49.4	51.3	68.3	68.5	57.1	63.3	46.9	65.5	57.7	45.4	58.7	56.3	52.4	50.3
Nutrient attributes of DM																								
CP content in DM	g kg^{-1} DM		131.4	132.4	132.1	132.1	347.9	70.7	100.1	68.8	74.4	95.4	88.0	168.1	53.5	250.0	221.0	129.0	201.0	211.0	114.0	227.0	201.0	109.0
ME content in DM	MJ kg^{-1} DM		10.1	10.2	10.2	10.2	11.7	6.9	10.3	5.1	6.0	6.7	9.4	9.2	8.1	8.9	6.4	9.6	8.7	5.9	9.2	8.4	6.9	8.1
Relative feed value (RFV)	-		93.7	94.3	94.2	94.2	128.7	62.2	91.6	47.6	55.1	63.0	83.2	90.1	69.2	96.2	73.8	89.0	89.6	68.9	85.0	89.9	75.8	75.1
Fresh matter ratio	ratio		1.2	1.7	1.4	1.3	1.1	1.1	1.2	1.2	1.2	1.3	2.9	2.8	3.6	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8	2.8
Crude lipid (XL)	g kg^{-1} DM		26.4	30.4	31.2	29.6	49.3	23.7	23.4	16.5	18.6	20.7	26.4	28.3	26.6	37.7	34.2	38.3	67.6	36.1	67.2	63.1	36.0	76.1
Crude fiber (XF)	g kg^{-1} DM		170.3	152.6	145.5	148.8	169.6	513.5	146.1	410.7	416.9	368.8	282.1	331.5	367.5	78.3	109.5	160.3	128.2	123.8	183.8	155.0	147.9	183.6
Crude ash (XA)	g kg^{-1} DM		57.4	56.6	60.2	57.4	64.9	46.2	50.1	128.2	150.6	107.1	110.3	127.5	73.9	88.0	76.5	66.8	118.0	108.0	84.2	106.9	115.2	111.1
N-free extractives (NFE)	g kg^{-1} DM		614.5	628.1	631.1	632.1	368.3	345.9	680.4	375.8	339.5	408.1	493.3	344.5	478.5	546.0	558.9	605.6	485.1	521.1	550.8	448.0	499.9	520.2
Gas production (Gb)	ml 200^{-1} mg^{-1} DM		51.2	51.2	50.9	51.1	49.7	30.7	54.0	18.1	24.1	28.3	47.5	42.5	39.3	35.4	19.2	45.5	29.4	15.8	37.4	27.3	23.6	26.1

Parameters

Daily milk yield	kg	6.0
Body mass	kg	400
Daily ME required	MJ	74.7
Daily CP required	g	845.2
Recommended daily DM intake	kg DM	7.5
Lactating	days	300
Milk price	USD kg^{-1}	0.42
Annual milk product	USD	751

Results

Fresh matter content, g	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10a	X11a	X12a	X10b	X11b	X12b	X10c	X11c	X12c
Run 1	143		909			125				-	-	-	-	-	-	-	-	-
Run 2	106		702									848	-	-	-	-	-	-
Run 3	84		927							-	-	-	368			-	-	-
Run 4	81		956							-	-	-	-	-	-	305		

		Run 1 Dry	Run 1 Fresh	Run 2 Dry	Run 2 Fresh	Run 3 Dry	Run 3 Fresh	Run 4 Dry	Run 4 Fresh
Ration weight	kg	7.4	8.7	7.3	12.2	7.4	10.1	7.4	9.9
Energy concentration	MJ kg^{-1}	10.1	8.6	10.2	6.1	10.2	7.4	10.2	7.6
Daily ME	MJ		74.7		74.7		74.7		74.7
Daily CP	g		971.5		971.5		971.5		971.5
Feed mixture cost	USD kg^{-1}	0.28	0.23	0.24	0.15	0.25	0.18	0.26	0.19
Daily feed mixture cost	USD		2.04		1.80		1.86		1.88
Annual feed mixture cost	USD		611.7		538.5		558.4		564.7
Annual profit from milk	USD		**138.9**		**212.1**		**192.1**		**185.8**

Sources: Menke et al., 1979; Dalakyan et al., 1980; Close and Menke, 1986; Model simulations

7.5 Discussion

7.5.1 SPAD-502 calibration

According to Peterson et al. (1993), the SPAD-502 readings are crop-specific thus necessitating a calibration for each crop and species. Although ample evidence underlined the suitability of the SPAD-502 for agricultural crops (Turner and Jund, 1991; Wood et al., 1992; Peng et al., 1993), this method has only sporadically been used for perennial vegetation (e.g. Moreau et al., 2004). The calibration of the SPAD-502 for the three deciduous tree species *E. angustifolia, G. triacanthos,* and *R. pseudoacacia,* resulted in high and positive coefficients of determination (R^2) between the SPAD-502 readings and leaf N contents for both linear and curvilinear relationships. This confirmed the utility of the SPAD-502 chlorophyll meter for a non-destructive and rapid field determination of tree foliar N (and indirectly of leaf CP) for the three tree species tested. Also, the relationships established could be effectively used year-round for the prediction of N and CP contents as long as the SPAD-502 readings remained within the calibrated range.

7.5.2 Crude protein contents as estimated with SPAD-502

Previous studies in the region pointed to the high feed quality of *E. angustifolia* as evidenced by its leaf CP contents of about 217 g CP kg^{-1} DM (Khamzina et al., 2006). This content is very much in line with the estimated CP values in this study that varied throughout the season between 200 and 250 g CP kg^{-1} DM. Reported CP contents of *R. pseudoacacia* leaves (Singh et al., 2002) amounted to 24% (240 g CP kg^{-1} DM), which also matched well with the range of 201 and 221 g CP kg^{-1} DM for this species in this study. In all simulations the assumed minimum level of dO of ~70% of the feed mix was achieved which is considered adequate for a medium (15 liters) to high (>20-30 liters) daily milk production (Close and Menke, 1986).

7.5.3 Achieving optimal feed diets

Previously conducted household surveys in Khorezm (Djanibekov, 2008) indicated that most feed for dairy consisted of locally produced, protein-deficient feedstuff, with CP:ME ratios of less than 13 g MJ^{-1} (Close and Menke, 1986). Furthermore, these surveys revealed that livestock keepers cannot reach a suitable CP:ME ratio by mixing available crops, crop residues and other byproducts. Many studies have discussed the advantages of including tree leaves into diary feeds (Devendra, 1992; Reddy and Elanchezhian, 2008) because the leaves of many tree species turned out to be, as in the present study, as nutritious as leguminous fodder crops and thus may offer a cheap source of proteins, although the results depended on the tree species.

Including tree leaves is one option to reach at least a nutritive feed with an adequate CP:ME ratio and hence enrich the poor diets as demonstrated by the simulation results. But in their quest to find an optimal feed diet for dairy cows livestock keepers in the study region are interested not only in the nutritive values of the diet components, but also in the costs involved. Although Devendra (1992) concluded that the inclusion of tree leaves as supplementary forage was financially beneficial in a number of countries, until today these feed resources have often been ignored also because livestock keepers are unaware of the best timing of including tree foliage and about the financial benefits such a choice may bring. This is true for Uzbekistan as well, where no up-to-date formulations of cost effective feeding rations for dairy cows exist. The results of the developed least-cost formulation LP model can be used to close this gap as the outcomes indicated the potential and scope of mixing the present dairy cow diets with tree foliage as to enrich these diets at the lowest cost, and whilst at least maintaining the present level of milk production. The foliage of *G. triacanthos* showed for example the highest financial potential for complementing the feed mix in May while concurrently reducing costs compared to the present feeding practices, whereas *E. angustifolia* leaves had the highest potential later in the growing season (July and September).

The elaborated model is thus used as an aid to demonstrate that the afforestation of marginal lands offers various benefits including the potential of adding tree foliage into the dairy feeding ration and at lower costs, which can be substantiated in the irrigated areas of Central Asia. For instance, when referring to about 37,000 ha of marginal land in the Khorezm region (Martius et al., 2004) and when assuming that this land could be converted to small tree plantations, due to the low foliage production in May and July the total amount would be too limited for feeding all cows (about 235,100 as of 2006) in the region during spring and summer. However, during this period of the growing season, livestock keepers have access to sufficient feedstuff (Djanibekov, 2006). The main bottleneck for satisfying the feed demand occurs in autumn, characterized by a deficit in green fodder and hence a supplement of the dairy feed rations with the tree leaves becomes most promising at that time. Assuming that the present marginal areas of ca. 37,000 ha would be planted equally to the three tree species examined, in September a total production of 27,721 ton DM of *E. angustifolia*, 765 ton DM of *R. pseudoacacia* and 9,900 ton DM of *G. triacanthos* can be expected for dairy cow feeding. With ca. 235,100 cows in the region (OBLSTAT, 2007), and when assuming a daily need of 74.7 MJ of ME and 845.2 g of CP for the production of 6 kg of 3.5% fat milk per day with a zero daily weight gain, a total of 350 ha of *E. angustifolia* would be needed to satisfy the monthly requirements of a least cost dairy feed ration in September taking into account the nutritive value of the foliage in this period. Thus, to satisfy the dairy feed needs during the feed deficit period (from November till May) assuming perfect storage conditions, the area necessary under afforestation should be at least 2,800 ha. Finally, the simulation findings indicated that livestock holders may rely more on cheaper wheat byproducts such as bran and less on cotton byproducts such as cottonseed cake, when dairy feed rations are supplemented with tree foliage.

At present the optimization model includes in particular information on CP, ME and the CP:ME ratio. However, in case additional information on the nutritive values of fodder would become available, a livestock keeper could include additional attributes of a feeding ration, e.g. CA, CF, CL and NFE, RFV or dO. Furthermore, the present findings are based on *in vitro* results, which still need to be confirmed by *in vivo* analyses. In particular the palatability of tree leaves is decisive. However, the analyses of the Gb with and without PEG did not indicate the presence of antinutritional factors in *E. angustifolia* and *G. triacanthos* such as tannins, which are known to limit the use of leaves as feed (Makkar, 1993). In contrast, *R. pseudoacacia* leaves may contain tannins which would render this species less suitable as dairy feed, despite the high CP contents, as is indicated also by the low dO values (45-56%) of this species (Table 7.4). But, although Devendra (1992) argued that the presence of tannins in feed does present a major barrier for utilization by cattle unless when used as supplementary feed (about 30% on a dry basis), *in vivo* analyses maybe needed to clarify this.

7.6 Conclusions

Following the calibration of the SPAD-502 for the three tree species, the outcomes confirmed the potential utility of this chlorophyll meter for a rapid determination of foliar N and thus also CP contents of the species *E. angustifolia*, *R. pseudoacacia* and *G. triacanthos*. The availability of permanent information on N and CP contents of tree leaves will benefit dairy producers in the irrigated drylands of Central Asia as to select the best time for feeding tree leaves. The simulation outcomes demonstrated that the inclusion of the CP-rich tree leaves into feed rations of dairy cows had not only a high potential to increasing the quality of the diets, but based on the nutritional values of the tree foliage the feed costs could be decreased by about half at the season-onset and by about one third at the mid-season and end of the season depending on the quality of the tree leaves that change during the growing season.

With the fodder prices observed in August of 2008, the cheapest dairy feed ration would consist of cottonseed husks, wheat bran and tree leaves. The harvest of *G. triacanthos* leaves in May and of *E. angustifolia* leaves in July and September, showed to be economically and nutritionally most attractive for those dairy producers facing a land deficit for fodder cultivation and livestock grazing. Thus, long-term investments in tree plantations on low-yielding or abandoned marginal cropland have substantial prospects for improving the income of farmers via improved livestock diets at lower costs than the present diets. Furthermore, following an afforestation of marginal cropland, protein-rich foliage can be gained and this could release pressure on natural pastures.

8 GENERAL DISCUSSION AND CONCLUSIONS

Trees are planted for socio-economic and/or ecological reasons. Although all tree species have the potential to serve several purposes, a judicious selection of species that best fits the needs of land users can increase the likelihood of achieving projected benefits. This is also true for afforesting degraded croplands in landscapes. To accomplish this we studied potential afforestation species in Uzbekistan including *Robinia pseudoacacia* L. (Black locust), which is a woody-legume benefiting from a *Rhizobium* symbiosis, and the non-legume *Elaeagnus angustifolia* L. (Russian Olive), which forms a symbiosis with *Frankia.* In case N_2 fixation occurs, such species provide ecological and economic benefits such as addition of nitrogen (N) to the soil via shed leaves, maintenance of soil fertility, availability of fixed N_2 for intercropped vegetation, rehabilitation of degraded soils and the production of considerable amounts of biomass. A combination of these potential benefits is the drive for the ecological restoration through the afforestation of salt-affected croplands in the irrigated areas of Central Asia.

Means were examined for converting marginal croplands (defined as areas which have lost their productive value) into forested areas from which various benefits can then be reaped. Through a field study, the impact of Phosphorus (P) amendments was analyzed on N_2 fixation by the N_2-fixers *R. pseudoacacia* and *E. angustifolia* against the reference species *Gleditsia triacanthos* L., and consequently, on growth and production of these nitrogen fixers. In addition, a lysimeter experiment was used to compare four different methods of quantifying N_2 fixation by *E. angustifolia*, which was the N_2-fixer with the highest potential in the field experiment, against the non-N-fixers *G. triacanthos* and *Ulmus pumila* L. The SPAD-502 chlorophyll meter, a non-destructive device to rapidly diagnose the leaf N concentration, was calibrated and validated for ten species including the species in the field and lysimeter trials. The practical value of this device is demonstrated by its use in the selection of the best time for gathering tree foliage as a supplement to livestock diets.

8.1 Phosphorus amendments for bolstering early growth and establishment

Compared to the nil treatment (no P amendments, 0-P), both P levels tested (high-P and low-P) were found to increase N_2 fixation by N_2-fixers measured with the ^{15}N

abundance method in the open field trial. The estimated N_2 fixation rates varied, however, according to the species and P rates applied. Whereas with high-P (the equivalent of 90 kg P ha^{-1}) N_2 fixation consistently and significantly increased over the three study years (2006-2008), with low-P (45 kg P ha^{-1}) the monitored boost in N_2 fixation was less consistent (section 3.4.3). These findings indicated that even higher P levels could further enhance N_2 fixation. Therefore, additional research should include trials with higher P amendments with the aim of identifying the most suitable P rate for enhancing N_2 fixation in resource-poor conditions.

Following the increase in N_2 fixation with high-P, biomass production of entire trees increased by 27% for *E. angustifolia* and by 57% for *R. pseudoacacia*. Furthermore, biomass of some individual tree fractions increased. For example foliage of *E. angustifolia* increased by 53% and stems of *R. pseudoacacia* by 118% (section 3.4.4). Compared to the nil treatment, however, the monitored differences in absolute biomass increments were statistically insignificant due to large coefficients of variation. This is in contrast to some findings in tropical areas (Luyindula and Haque, 1992; Sanginga, 2003), but has been corroborated by others (Binkley et al., 2003). Possibly, more repetitions should be included in the experimental designs to confirm whether N_2 fixation significantly boosts absolute biomass production in the early growth stages. This is of particular interest because the findings from the analyses of relative growth rates illustrated that various growth parameters (section 3.4.6) increased with high-P, although these impacts differed between N_2-fixers. High-P significantly affected relative differences in height, diameter, unit production rate and nitrogen productivity. These differences were not discerned in standard absolute growth analyses. Hence, absolute and relative growth analyses must be performed together to gain an overall understanding of growth performance. N_2 fixation rates were sufficient to satisfy the N demand of both N_2-fixers. Amounts of N_2 fixed in the open field trial were in line with previously reported findings (section 3.5.1). Hence, although N_2 fixation was quantified here against one reference species only, the results appeared to be within the commonly observed range and are likely accurate. However, future research designs should include more than one suitable reference species to increase the probability of accurate quantification of N_2 fixation. When using only one reference species, the correct choice of the reference species is critical. Present research findings indicated that *G.*

triacanthos could be such a suitable reference species (section 3.5.1). However some reports suggest that this species may fix N_2 through non-nodule N_2 fixation under very specific environmental conditions (Bryan, 1995). Previous field research (Khamzina et al., 2009a) indicated that *Ulmus pumila* L. is a suitable reference species for estimating the amount of N_2 fixed by *E. angustifolia*.

In addition to the difficulties with selecting multiple reference species, the absence of an accurate *B* value complicated the quantification of N_2 fixation in open field trials with the ^{15}N abundance method. The *B* value indicates the N_2 fixation potential of species in an N-free growth environment. The range of *B* values previously reported for N_2-fixers stresses the importance of using accurate *B* values. Where *B* values are unknown, it is a common practice to substitute the absent *B* value with a literature value. Whereas much information exists on *Rhizobium*-based symbioses, little research has been done on *Frankia* associations. Unfortunately, the determination of these values for *Frankia*-non-legume associations requires intensive laboratory-supported studies and analyses, which fell beyond the scope of the present study. As long as *B* values are non-existent, as is the case for *E. angustifolia* and *R. pseudoacacia* under the growth conditions examined, a combination of N_2 fixation quantification methods increases the confidence of the estimated measures.

8.2 Lysimeter-based experiment and N_2 fixation quantifications

Quantification of N_2 fixation in tree stands can be hampered by difficulties associated with collecting all biomass from older trees grown in a (dense) stand. Although spacing within the sample rows and between field plots was chosen to avoid these well-known stumbling blocks, a lysimeter-based experiment was simultaneously conducted to verify and increase the accuracy of quantifying N_2 fixation. Using the same spacing as in the field experiment, N_2 fixation by *E. angustifolia*, which showed the highest N_2 fixation rates in the open field trial (section 3.3.3), was measured against two reference species: *G. triacanthos* and *U. pumila*, using the ^{15}N enrichment technique (^{15}NET). The experiment (section 4.2) was designed also to compare four commonly used methods to quantify N_2 fixation (Chapter 5). In addition to the ^{15}NET, N_2 fixation was quantified by ^{15}N abundance (^{15}NA), N difference (ND) and A-value (AV) methods. Due to logistical reasons, these verification studies were not extended to *R. pseudoacacia,* for which few reference values for Central Asian regions are available, either.

The use of the ^{15}NET in lysimeters showed high accuracy and reliability with one-year-old trees while using low doses of enriched ^{15}N fertilizer (section 4.3). However, for two-year-old trees the amount of ^{15}N fertilizer added was insufficient to ensure proper growth of both reference species. Therefore, in the second year higher amounts of ^{15}N fertilizer were applied to the reference species, which overcame this limitation, and biological N_2 fixation (BNF) estimates were possible using the AV method. A comparison of the results for the four N_2 quantification methods illustrated the suitability of using lysimeters for trees when using the carefully designed system for applying enriched ^{15}N fertilizer. The highest accuracy was found with the AV method, but financial and material considerations may favor the total ND method (section 5.3). The latter has clear implementations for the poorly endowed research institutions in Central Asia. When using a similar methodological set up (section 5.3), ND can be used to estimate N_2 fixation rates without expensive ^{15}N enriched materials. Furthermore, the simple irrigation technique of using a polyethylene pipe in a lysimeter, through which water and labeled ^{15}N fertilizer could be supplied from below, effectively ensured a uniform distribution of the ^{15}N fertilizer throughout the soil profile.

8.3 The use of SPAD-502 for within-season management of trees

Chemical analyses of tree fractions elucidated that leaf N concentrations of both N_2-fixers in the open field trial were higher than those of the reference species. The N concentration of the foliage of both N_2 fixers was indirectly bolstered by the application of P amendments that had triggered the increase in N_2 fixation. Thus although the monitored increase in leaf biomass was statistically insignificant (appendix 10.2), the resulting increase in leaf N concentration due to high-P was significant for *E. angustifolia*. Low-cost, easy to use, and rapid diagnostic tools that allow land users to monitor near real-time tree N dynamics can improve tree and plantation management and explore the benefits of higher leaf N concentrations. In Uzbekistan, this is particularly advantageous because the present forest administration has insufficient means and near real-time information to manage many types of tree plantings (Tupitsa, 2010). During 2006-2008, an optical sensor-based chlorophyll meter (SPAD-502) was therefore tested, calibrated, and validated for the three species included in the field experiments (section 6.3.1). The temporal and spatial validation of SPAD-502 estimates for the species in the core experiments showed very high correlations with the chemically analyzed values. This indicated that as long as the measured values fall within the range of the SPAD-502 indices monitored during calibration and validation, the established relationships are adequate for monitoring leaf N status.

Optical sensor meters are reputed as an N management tool, in particular for annual but also for perennial vegetation. However, because the mineral N makes up about 16% (Menke et al 1979) of the leaf crude protein (CP), which is an important indicator for feed quality, the established relationships between N/CP contents and SPAD-502 readings can also be used also for selecting the best time for harvesting tree foliage. The profitability of including tree foliage in the feed diets of cattle was simulated with a specially elaborated least-cost-ratio model (section 7.4). The findings showed that the leaves of non-fixer *G. triacanthos* would be better harvested in May, but for the N_2-fixer *E. angustifolia* July or September would be better. The model simulations revealed that the cheapest dairy feed ration would consist of cottonseed husks, wheat bran, and tree leaves. Although the leaves of N_2-fixer *R. pseudoacacia* contained high N/CP contents, their use as supplementary fodder is probably limited due to the high amounts of tannins. Further *in vivo* research should be conducted to clarify/verify the feed value of the studied species. In case confirmed, tree plantations on marginal cropland can contribute to the production of protein-rich feed and could therefore relax the on-going pressure on natural grazing lands.

8.4 Overall conclusions

The results of the present study show that afforestation with the N_2-fixing tree species can be self-sufficient in N through their N_2 fixation ability on nutrient-exhausted croplands. They would enhance soil fertility of managed landscapes. Planting N_2-fixing tree species such as actinorhizal *E. angustifolia* and leguminous *R. pseudoacacia* could bring ecological and economic benefits through:

(1) increasing the juvenile growth and N contribution to the soil-plant system via enhanced N_2 fixation rates by localized applications of P;

(2) increasing the productive capacity of the degraded landscapes via production of large amounts of biomass which can be used as fuelwood or construction material;
(3) improving livestock nutrition by supplementing the common feed with high-quality tree foliage; with the calibrated diagnostic tool, the N/CP dynamics of the foliage can now be monitored in near real-time and support the selection of the best time for collecting tree foliage for supplementation. Hence, following the afforestation of marginal cropland, protein-rich foliage can be gained for feeding livestock without competing with crops and while easing the pressure on natural grazing lands.

Afforestation is a recognized land use option to support ecological restoration of degraded landscapes or marginal, salt affected croplands. This was confirmed by the findings in this study conducted in the lower regions of the Amu Darya River. The findings furthermore showed that P applications at an early stage of afforestation and the use of optical sensors during the growing season have large potential for improving management of trees and tree plantations. The profitability of these management techniques had not been confirmed, but given the prospects of annual recurring returns (Lamers et al., 2008) from tree plantations in dryland areas, it is at present very likely that investments in these techniques could be recovered in little time. Afforestation of marginal croplands will benefit nature and humans. At present, there is a need for administrators and land users to become familiar with (i) the principle of afforesting marginal cropland and (ii) the fact that such land use is not to be seen as a rival to out-compete annual crop production, but rather as a useful complement to combat on-going land degradation and inefficient use of resources. Since 2011 was declared the International Year of Forests, the GOU has called a nationwide appeal for tree plantings (Figure 1.1). By accepting afforestation as a means of improving degraded croplands, Uzbekistan has the opportunity to set a precedent within Central Asia, where many similar agro-ecological landscapes exist.

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10 APPENDICES

Appendix 10.1: Concentrations of total N (%), available P and exchangeable K (mg kg^{-1}) in 1 m soil layer according to tree species and P treatments at the onset and end of each growing season. The initial soil data is excluded. ± indicate standard deviation of means

Parameter				**N (%)**		
Species	P treatment	Oct 2006	May 2007	Oct 2007	May 2008	Oct 2008
E. angustifolia	0-P	0.4 ± 0.1	0.4 ± 0.2	0.5 ± 0.1	0.4 ± 0.2	0.4 ± 0.1
	Low-P	0.3 ± 0.1	0.4 ± 0.2	0.5 ± 0.1	0.3 ± 0.2	0.4 ± 0.1
	High-P	0.4 ± 0.1	0.4 ± 0.2	0.5 ± 0.1	0.4 ± 0.2	0.5 ± 0.1
	Overall means	**0.4 ± 0.1**	**0.4 ± 0.2**	**0.5 ± 0.1**	**0.4 ± 0.2**	**0.5 ± 0.1**
G. triacanthos	0-P	0.3 ± 0.1	0.4 ± 0.2	0.5 ± 0.2	0.5 ± 0.2	0.4 ± 0.2
	Low-P	0.3 ± 0.1	0.4 ± 0.2	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.2
	High-P	0.3 ± 0.1	0.4 ± 0.2	0.4 ± 0.2	0.4 ± 0.1	0.4 ± 0.2
	Overall means	**0.3** ± 0.1	**0.4 ± 0.2**	**0.4 ± 0.2**	**0.5 ± 0.1**	**0.4 ± 0.2**
R. pseudoacacia	0-P	0.3 ± 0.1	0.4 ± 0.2	0.4 ± 0.2	0.5 ± 0.1	0.4 ± 0.1
	Low-P	0.3 ± 0.1	0.4 ± 0.2	0.5 ± 0.1	0.5 ± 0.1	0.4 ± 0.1
	High-P	0.3 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.4 ± 0.2
	Overall means	**0.3 ± 0.1**	**0.4 ± 0.2**	**0.5 ± 0.1**	**0.5 ± 0.1**	**0.4 ± 0.1**
				ANOVA, probability > F(=α)		
Time				0.044		
Species				0.826		
P treatment				0.594		
Time*species				0.172		
Time*P treatment				0.941		
Species*P treatment				0.771		

Parameter				**Available P_2O_5** (mg kg^{-1})		
Species	P treatment	Oct 2006	May 2007	Oct 2007	May 2008	Oct 2008
E. angustifolia	0-P	10.5 ± 6.9	14.3 ± 8.6	10.6 ± 7.5	18.4 ± 14.2	10.4 ± 5.9
	Low-P	21.6 ± 18.6	9.3 ± 3.9	10.1 ± 5.1	12.1 ± 10.1	13.6 ± 6.2
	High-P	14.9 ± 10.4	13.7 ± 9.5	12.1 ± 4.5	10.2 ± 5.9	6.7 ± 1.9
	Overall means	**15.6 ± 11.3**	**12.4 ± 7.5**	**10.9 ± 5.5**	**13.6 ± 11.4**	**10.2 ± 5.5**
G. triacanthos	0-P	3.7 ± 1.8	10.8 ± 5.1	6.0 ± 3.2	6.6 ± 3.4	5.3 ± 1.7
	Low-P	8.5 ± 2.3	9.3 ± 4.8	5.9 ± 4.3	7.6 ± 5.0	13.2 ± 9.3
	High-P	13.2 ± 2.6	10.6 ± 5.3	5.6 ± 3.2	8.6 ± 6.7	10.1 ± 5.1
	Overall means	**8.5 ± 4.5**	**10.2 ± 4.8**	**5.8 ± 3.3**	**7.4 ± 4.7**	**10.4 ± 6.9**
R. pseudoacacia	0-P	7.5 ± 3.9	8.6 ± 5.8	4.2 ± 2.9	11.8 ± 7.4	8.1 ± 3.6
	Low-P	14.4 ± 1.1	7.5 ± 3.0	8.5 ± 6.3	22.1 ± 8.0	11.2 ± 5.4
	High-P	13.1 ± 10.1	9.1 ± 5.4	5.4 ± 3.3	15.9 ± 2.6	7.8 ± 4.4
	Overall means	**11.7 ± 6.6**	**8.4 ± 4.6**	**6.1 ± 4.5**	**16.7 ± 8.2**	**8.5 ± 4.3**
				ANOVA, probability > F(=α)		
Time				0.002		
Species				0.001		
P treatment				0.108		
Time*species				0.050		
Time*P treatment				0.211		
Species*P treatment				0.525		

Appendix 10.1: continued

Parameter		K_2O (mg kg^{-1})				
Species	P treatment	Oct 2006	May 2007	Oct 2007	May 2008	Oct 2008
E. angustifolia	0-P	120 ± 13	138 ± 65	133 ± 24	116 ± 36	86 ± 37
	Low-P	97 ± 16	67 ± 24	87 ± 16	86 ± 35	55 ± 11
	High-P	131 ± 21	75 ± 25	103 ± 25	102 ± 36	75 ± 34
	Overall means	**116 ± 22**	**94 ± 51**	**108 ± 28**	**101 ± 37**	**72 ± 30**
G. triacanthos	0-P	136 ± 49	70 ± 14	83 ± 26	78 ± 23	83 ± 37
	Low-P	101 ± 13	89 ± 30	96 ± 31	95 ± 41	77 ± 31
	High-P	105 ± 25	73 ± 17	100 ± 36	97 ± 40	107 ± 52
	Overall means	**114 ± 34**	**77 ± 21**	**93 ± 30**	**89 ± 34**	**93 ± 45**
R. pseudoacacia	0-P	108 ± 29	120 ± 81	94 ± 24	106 ± 35	82 ± 38
	Low-P	90 ± 10	66 ± 25	91 ± 27	131 ± 41	65 ± 31
	High-P	69 ± 13	120 ± 27	117 ± 45	108 ± 41	69 ± 24
	Overall means	**89 ± 24**	**102 ± 55**	**100 ± 33**	**117 ± 39**	**73 ± 31**
				ANOVA, probability > F(=α)		
Time				<0.0001		
Species				0.582		
P treatment				0.191		
Time*species				0.021		
Time*P treatment				0.341		
Species*P treatment				0.062		

Appendix 10.2: Least square means of the dry matter production (t ha^{-1}) separated into fractions, above and below ground and total biomass as affected by species, phosphorus application and year interactions. Within one column, means followed by the same letter are not significantly different at $P<0.1$ according to the Tukey post-hoc test

Interaction/Variable		Leaves	Twigs	Stem	Coarse roots	Fine roots	Stump	Above ground	Below ground	Total biomass
		Dry matter production, t ha^{-1}								
Year*Species										
2006	*Elaeagnus angustifolia*	0.97 a	1.45 a	0.55 a	0.70 a	0.33 a	na	2.97 a	1.02 a	4.00 a
	Gleditsia triacanthos	0.02 c	0.11 c	0.01 b	0.19 c	0.04 c	na	0.14 c	0.23 c	0.37 c
	Robinia pseudoacacia	0.16 b	0.26 b	0.04 b	0.34 b	0.13 b	na	0.45 b	0.46 b	0.92 b
	P value	<0.001	<0.001	<0.001	<0.001	<0.001	-	<0.001	<0.001	<0.001
2007	*Elaeagnus angustifolia*	5.38 a	12.60 a	8.17 a	2.76 a	0.39 a	2.48 a	28.62 a	3.15 a	31.77 a
	Gleditsia triacanthos	0.22 c	0.31 c	0.04 c	0.12 c	0.06 b	0.52 c	1.07 c	0.18 c	1.25 c
	Robinia pseudoacacia	2.00 b	2.53 b	1.66 b	0.99 b	0.25 c	1.26 b	7.44 b	1.24 b	8.68 b
	P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
2008	*Elaeagnus angustifolia*	15.34 a	35.77 a	50.12 a	7.27 a	0.29 a	8.92 a	111.16 a	7.55 a	117.71 a
	Gleditsia triacanthos	0.16 c	0.61 c	0.19 c	0.16 c	0.04 c	0.59 c	1.57 c	0.20 c	1.77 c
	Robinia pseudoacacia	3.29 b	5.68 b	5.02 b	3.05 b	0.10 b	2.38 b	16.38 b	3.14 b	19.52 b
	P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Overall	***P* value**	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.11**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Year*P treatment										
2006	0-P	0.36 a	0.57 a	0.19 a	0.44 a	0.19 a	na	1.12 a	0.63 a	1.75 a
	Low-P	0.37 a	0.52 a	0.10 a	0.39 a	0.13 a	na	0.99 a	0.52 a	1.51 a
	High-P	0.37 a	0.65 a	0.27 a	0.37 a	0.16 a	na	1.30 a	0.53 a	1.83 a
	P value	0.99	0.76	0.95	0.96	0.49	-	0.75	0.87	0.75
2007	0-P	3.05 a	5.72 a	3.68 a	1.62 a	0.24 a	1.40 a	13.86 a	1.85 a	15.71 a
	Low-P	1.99 a	4.30 a	2.69 a	1.15 a	0.25 a	1.23 a	10.19 a	1.40 a	11.55 a
	High-P	2.42 a	4.90 a	3.18 a	1.02 a	0.21 a	1.59 a	12.09 a	1.22 a	13.31 a
	P value	0.44	0.73	0.75	0.44	0.82	0.46	0.66	0.44	0.63
2008	0-P	4.83 a	13.59 a	16.91 a	4.31 a	0.19 a	4.14 a	39.46 a	4.47 a	43.94 a
	Low-P	6.51 a	14.75 a	18.27 a	2.69 a	0.13 a	3.43 a	40.96 a	2.83 a	43.76 a
	High-P	7.65 a	16.27 a	20.86 a	3.66 a	0.16 a	4.48 a	49.26 a	3.79 a	53.05 a
	P value	0.58	0.80	0.96	0.47	0.71	0.44	0.79	0.42	0.81
Overall	***P* value**	**0.61**	**0.94**	**0.99**	**0.63**	**0.83**	**0.79**	**0.91**	**0.63**	**0.92**

Appendix 10.2: continued

Interaction/Variable		Leaves	Twigs	Stem	Coarse roots	Fine roots	Stump	Above ground	Below ground	Total biomass
Species*P treatment		Dry matter production, t ha^{-1}								
E. angustifolia	0-P	6.15 a	16.47 a	18.30 a	4.34 a	0.37 a	6.16 a	45.03 a	4.71 a	49.73a
	Low-P	7.24 a	15.02 a	19.67 a	3.24 a	0.37 a	4.59 a	45.12 a	3.61 a	48.73 a
	High-P	9.42 a	20.72 a	24.76 a	3.46 a	0.25 a	7.24 a	59.43 a	3.70 a	63.30 a
	P value	0.51	0.72	0.79	0.68	0.18	0.29	0.73	0.61	0.77
	Difference[a]	**53%**	**26%**	**35%**	**-20%**	**-32%**	**18%**	**32%**	**-21%**	**27%**
G. triacanthos	0-P	0.11 b	0.27 b	0.3 a	0.13 a	0.04 b	0.47 b	0.70 b	0.17 a	0.87 b
	Low-P	0.08 ab	0.24 b	0.7 a	0.18 a	0.04 b	0.52 ab	0.74 ab	0.22 a	0.95 ab
	High-P	0.20 a	0.50 a	0.14 a	0.16 a	0.06 a	0.66 a	1.28 a	0.22 a	1.50 a
	P value	0.02	0.02	0.89	0.31	0.05	0.06	0.06	0.16	0.03
	Difference [a]	**81%**	**85%**	**-53%**	**23%**	**50%**	**40%**	**82%**	**29%**	**72%**
R. pseudoacacia	0-P	1.54 a	2.14 a	1.44 a	1.60 a	0.16 ab	1.33 b	6.01 a	1.76 ab	7.77 a
	Low-P	1.83 a	2.91 a	2.14 a	0.92 a	0.12 b	1.87 ab	8.13 a	1.04 b	9.17 a
	High-P	2.07 a	3.42 a	3.14 a	1.86 a	0.19 a	2.26 a	10.13 a	2.05 a	12.19 a
	P value	0.63	0.37	0.56	0.21	0.10	0.03	0.39	0.11	0.40
	Difference[a]	**34%**	**60%**	**118%**	**16%**	**19%**	**70%**	**69%**	**16%**	**57%**
Overall	***P* value**	**0.89**	**0.96**	**0.99**	**0.53**	**0.02**	**0.54**	**0.97**	**0.36**	**0.99**

[a] *Differences in dry matter accumulation in different tree fraction of high-P compared to 0-P treatment*

Appendix 10.3: Concentration of exchangeable cations in 1 m soil layer. ± indicate standard deviation of means

		Cation content, cmol(+) kg^{-1}								
		October 2006			October 2007			October 2008		
Species	P treatment	Na^+	Ca^{2+}	Mg^{2+}	Na^+	Ca^{2+}	Mg^{2+}	Na^+	Ca^{2+}	Mg^{2+}
	0-P	1.61 ± 0.11	1.68 ± 0.60	0.95 ± 0.15	5.87 ± 4.54	2.90 ± 2.35	2.01 ± 1.39	5.82 ± 5.01	5.15 ± 2.27	3.50 ± 2.92
E. angustifolia	Low-P	1.97 ± 0.52	1.36 ±0.40	1.15 ± 0.27	0.79 ± 0.26	1.04 ± 0.34	0.53 ± 0.54	4.93 ± 3.95	3.65 ± 2.29	1.90 ± 1.03
	High-P	3.38 ± 0.34	2.44 ± 0.86	2.01 ± 0.56	5.51 ± 3.81	3.33 ± 1.45	2.53 ± 1.37	4.25 ± 2.22	7.25 ± 3.23	2.90 ± 1.07
	Overall means	**2.32 ± 0.34**	**1.82 ± 0.76**	**1.37 ± 0.59**	**4.06 ± 3.97**	**2.42 ± 1.81**	**1.69 ± 1.38**	**5.00 ± 3.67**	**5.35 ± 3.00**	**2.77 ± 1.88**
	0-P	1.84 ± 0.42	1.86 ± 0.68	1.15 ± 0.52	2.94 ± 2.53	2.16 ± 1.88	1.38 ± 1.50	2.00 ± 0.99	1.94 ± 1.11	1.44 ± 0.56
G. triacanthos	Low-P	1.53 ± 0.42	1.26 ± 0.32	1.11 ± 0.45	1.20 ± 1.05	1.52 ± 1.27	0.83 ± 0.65	1.91 ± 1.87	2.25 ± 2.21	1.32 ± 1.30
	High-P	1.40 ± 0.45	1.48 ± 0.62	1.19 ± 0.42	3.30 ± 3.34	2.78 ± 1.79	1.68 ± 1.43	2.20 ± 1.40	2.89 ± 2.04	1.94 ± 0.91
	Overall means	**1.59 ± 0.44**	**1.53 ± 0.58**	**1.15 ±0.43**	**2.54 ± 2.52**	**2.15 ± 1.63**	**1.30 ± 1.22**	**2.08 ± 1.37**	**2.49 ± 1.86**	**1.66 ± 0.95**
	0-P	1.74 ± 0.64	1.38 ± 0.55	0.95 ± 0.46	3.32 ± 2.87	2.04 ± 2.06	1.38 ± 0.81	3.74 ± 3.77	3.23 ± 2.52	1.76 ± 1.38
R. pseudoacacia	Low-P	1.35 ± 0.50	1.34 ± 0.63	0.79 ± 0.29	1.20 ± 0.99	2.28 ± 2.39	1.21 ± 1.15	2.49 ± 2.28	2.78 ± 2.16	1.39 ± 0.98
	High-P	2.12 ± 0.67	1.78 ± 0.41	1.05 ± 0.42	2.13 ± 1.98	1.28 ± 1.09	0.81 ± 0.62	3.26 ± 2.73	2.69 ± 1.97	1.67 ± 1.02
	Overall means	**1.74 ± 0.65**	**1.50 ± 0.54**	**0.93 ± 0.38**	**2.22 ± 2.13**	**1.86 ± 1.83**	**1.13 ± 0.86**	**3.16 ± 2.93**	**2.90 ± 2.17**	**1.61 ± 1.11**

Appendix 10.4: Overviews of the experimental site in (a) April, 2006, (b) May, 2007, and (c) July, 2008

a)

b)

c)

Appendix 10.5: Detached and washed root nodules of the three-year-old *E. angustifolia* (a), and root nodules of the three-year old *R. pseudoacacia*, both collected during root excavations (b)

a)

b)

Appendix 10.6: Overview of the lysimeter trial; (a) plastic bottles with water and ^{15}N enriched fertilizer prepared for application to the soil in each lysimeter, (b) trees growing in lysimeters

a)

b)

Appendix 10.7: SPAD-502 measurements on *R. pseudoacacia* tree leaves

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