The influence of nurse shrubs on growth and establishment of *Olea europaea subsp. cuspidata*

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Abstract
Ethiopia is rich in biodiversity but due to poverty biodiversity is declining, which further threatens rural livelihoods. *Olea europaea subsp. cuspidata* is a native multipurpose tree, which has declined due to over use. A restoration of this species would serve biodiversity conservation and improve the livelihood of the rural population.

Nurse shrubs are shrubs that can facilitate the growth and establishment of other individuals; therefore they can be a useful tool for forest restoration.

The aim of the study was to find out whether *Olea europaea subsp. cuspidata* occurs with nurse shrubs and - if so – whether these nurse shrubs influence *Olea europaea subsp. cuspidata* in regard to improved mycorrhization and nitrogen supply.

We took leaf samples and recorded the shrubs species occurring within the double average crown size radius of every third *Olea europaea subsp. cuspidata* along the path. Further we took leaf and root samples of *Olea europaea subsp. cuspidata* and recorded their growth. The leaf samples were analyzed for total leaf nitrogen and $^{15}$N content. The root samples were stained; we then counted vesicles, hyphae and arbuscules to assess the mycorrhization.

The most common shrubs within the double average crown size radius were *Dodonea angustifolia*, *Carissa edulis* and *Clutia spec.* *Olea europaea subsp. cuspidata* associated with other shrubs were significantly taller than not-associated individuals, but there were only very small to no differences in mycorrhization or nitrogen content between the groups, even though the number of vesicles and the nitrogen content were correlated to plant height.

Not-associated *Olea europaea subsp. cuspidata* are most likely smaller than associated individuals, because they are limited, not as we thought by mycorrhization or nitrogen supply, but by browsing. *Olea europaea subsp. cuspidata* associated with shrubs have a decreased browsing pressure and therefore grow better. This suggests that the influence of nurse shrubs on *Olea europaea subsp. cuspidata* is not species specific but general.
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1. Why we should restore ecosystems

1.1. Ecosystem services and human well-being

“Ecosystem services are the benefits people obtain from ecosystems.” They are composed of provisioning, regulating and cultural services which directly affect people, while supporting services maintain the other three services ([Fig. 1](#)) (Millennium Ecosystem Assessment, Reid 2005).

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<tr>
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Supporting Services

*Services necessary for the production of all other ecosystem services*

- Soil formation
- Nutrient cycling
- Primary production

![Fig. 1 Ecosystem services (Millennium Ecosystem Assessment, Reid 2005)](image)

Every part of Earth produces a bundle of ecosystem services. Human intervention, such as agriculture, can increase some services, like food production, which often comes at the expense of other services, such as water regulation (Millennium Ecosystem Assessment, Reid 2005; Hooper et al. 2005). Depending on the magnitude of human intervention and the properties of the given ecosystem human intervention can initiate a process of degradation (Moges, Holden 2009). Along with the state of degradation ecosystem services decline, while time and cost for regeneration rise (Chazdon 2008). Therefore degradation compromises the output of provisioning services necessary for sustaining livelihoods.

The output of provisioning services does not reflect their condition, since a given output may not be sustainable over the long term. The output depends on the output and the “stock” of the good, so in the long term, the production of over harvested resources will fall.
As long as manufactured capital can compensate for losses of the natural capital the production can be maintained, but once a critical level is reached, the output will decline, the ecosystem has been degraded (Millennium Ecosystem Assessment, Reid 2005). Individuals and governments can buffer the availability of ecosystem services against natural variation. Substitution is possible for some ecosystem services, but often the cost of a technological substitution is high, temporary and might not replace all the services lost (Millennium Ecosystem Assessment, Reid 2005; Hooper et al. 2005; Chazdon 2013). Another problem is that the individuals gaining the benefits are not those who originally benefited from the ecosystem services (Millennium Ecosystem Assessment, Reid 2005).

Fig. 2 Ecosystem services and human well-being (Millennium Ecosystem Assessment, Reid 2005)

The provisioning, regulating and cultural function of ecosystem services supplies goods and other services which sustain various aspects of human well-being (Fig. 2) (Millennium Ecosystem Assessment, Reid 2005). Locals can use derived services, e.g. forest coffee, directly for household consumption and as a source of cash income (Melaku et al. 2014). Especially the rural poor reduce their vulnerability through diverse and complex mixes of activities which draw on ecosystem services. Changes to ecosystems thus do not only impair the system itself but can also have immediate and lagged impacts on the population.
(Millennium Ecosystem Assessment, Reid 2005). For example a decrease in forest cover leads to the decrease of non-timber forest products supporting the livelihoods of rural poor, which results in even further impoverished livelihoods.

### 1.2. Biodiversity loss

As discussed in the previous chapter ecosystem services are vital for human livelihoods. Biodiversity is an important factor for assessing and preserving ecosystem properties which underlie the output of ecosystem services.

Red List Data shows that the survival of amphibians, birds and mammals in different biogeographic realms is declining. Although the status of some species has improved (32 in total), many more are more likely to go extinct and the expected rate of species extinction is unchanged or even accelerating. Different groups, taxa and geographic regions vary in their overall level of threat and rate of deterioration. The major threat across groups is habitat loss/habitat conversion. Other threats are utilization, direct mortality and pollution (Silva et al. 2009).

Species functional characteristics influence ecosystem properties and operate in a variety of contexts like effects of dominant species, keystone species, ecological engineers and interaction among species. The effects of species loss or changes in composition and the mechanisms by which the effects become visible can differ widely (Hooper et al. 2005). Some systems might suffer severely from the loss of any species because most of its species essentially contribute to its productivity (rivet hypothesis). Other systems are less sensitive to species loss, because only a few keystone species contribute to the productivity of the given ecosystem (redundant species hypothesis) (van Andel, Aronson 2012).

Therefore some ecosystem properties are initially insensitive to species loss because (a) the given system has multiple species carrying out similar functional roles, (b) some species contribute little to ecosystem properties and (c) properties may primarily controlled by abiotic environmental conditions.

Certain combinations of species are can be complementary in their patterns of resource use and can increase average rates of productivity and nutrient retention, but the identification of which and how many species act complementary in complex communities is just beginning.

On the ecosystem-level even the disappearance of rare species can have a strong impact on the ecosystem (Hooper et al. 2005). Therefore, while some resources for human livelihoods are still provided, the ongoing provision of those resources in a degraded ecosystem can be
limited or insecure (Griscom, Ashton 2011; Millennium Ecosystem Assessment, Reid 2005). The loss of particularly well adapted species, due to over use, might result in a serious decline in productivity at the most climate stressed sites (Eshete et al. 2011).
2. **How can we restore ecosystems**

‘Ecological restoration is the process of assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed’ (Society for Ecological Restoration International 2004). Restoring ecosystems can cause severe economic restrictions because it requires income reductions (fewer livestock) and expenditures for vegetation manipulation such as seeding, burning, herbicide treatments, or selective plant removal (Whisenant 2001). However, as discussed in previous chapters, ecosystems and their services provide natural capital for human livelihoods, therefore not restoring degraded ecosystems can set off a cycle of poverty.

2.1. **From degradation to restoration**

‘Degradation (1) reduces the number of desired plant and animal species; (2) reduces plant biomass; (3) decreases primary production; (4) reduces energy flow to grazing and decomposer components of the food chain; (5) depletes macronutrient pools and (6) reduces soil stability’ (Whisenant 2001).

![Fig. 3 Stepwise degradation of vegetation illustrating the two common transition thresholds (Whisenant 2001)]
needed to cross further thresholds declines and without assistance a degraded ecosystem might not recover fully (van Andel, Aronson 2012; Whisenant 2001). Therefore, to enable recovery, those thresholds, controlled by biotic and abiotic limitations need to be addressed by restoration efforts (Fig. 3) (Hobbs, Suding 2009; Whisenant 2001).

The ability to regenerate without assistance after degradation depends on the species adaptations to abiotic factors, past land use type and intensity, the surrounding habitat and disturbance history. These factors affect seed rain, regeneration from within site, seed germination, seedling survival and seedling growth and therefore determine the dynamics of colonization and establishment (Fig. 4) (Griscom, Ashton 2011; Holl 2007).

An ecosystem counts as restored when it contains sufficient biotic and abiotic resources to continue its development without further assistance or subsidy (Society for Ecological Restoration International 2004). The following attributes provide a basis for evaluating the ability of restored ecosystems to react to normal ranges of environmental stress and disturbance.

Ideally, restored or functional ecosystems show the following features

1. Characteristic assemblage of species which occur in the reference ecosystem
2. Indigenous individuals to the greatest practicable extent
3. All functional groups for the continued development are present
4. The physical environment sustains reproducing populations of species necessary for continued stability
5. Normal function according to the ecological state of development
6. Integration into a larger ecological matrix with which it interacts
7. Potential threats to integrity are reduced as much as possible
8. Resilience against normal periodic stress
9. Potential to persist indefinitely under existing environmental conditions

(Society for Ecological Restoration International 2004)

2.2. Restoration of forests

Plants in tropical forests can regenerate from propagules within the site or dispersed from outside sources. This includes seeds from the seedbank, seedlings established at the time of land abandonment or resprouting from roots or stems (Holl 2007). “Depending on the degradation of an initially forested ecosystem, a range of management activities can at least partially restore levels of biodiversity and ecosystem services given adequate time (years) and financial investment (capital, infrastructure and labor)” (Fig. 5). The outcomes of particular approaches are (1) restoration of soil fertility for agricultural or forestry use, (2) production of timber and non-timber forest products, or (3) recovery of biodiversity and ecosystem services (Chazdon 2008).

![The restoration staircase (Chazdon 2008)](image)

**Fig. 5 The restoration staircase (Chazdon 2008)**

2.2.1. Nurse shrubs in forest restoration

Nurse shrubs are shrubs which facilitate the growth and establishment of another individual. This relationship can be species specific and, depending on the system, positive effects can overrule negative effects of competition (Holmgren, Scheffer 2010; Høgh-Jensen, Schjoerring...
This facilitation can be provided in numerous ways, affecting the influx of propagules, germination, establishment, browsing and nutrition.

Nurse shrubs can provide structures for seed predators which can improve seed rain via increasing seed density and species richness of seeds deposited on a given area (Duncan, Chapman 1999). Further seedlings can have an increased chance for germination and survival when planted under nurse shrubs instead of bare soil patches. This effect can be accounted to various facilitation effects such as protection from drought stress, high irradiation, deposition or low browsing pressure, (Aerts et al. 2007; Aerts et al. 2006a; Boehm 2011).

Plant canopies can ameliorate transpiration rates by providing cooler and moister air (Holmgren, Schefter 2010). At low browsing pressures seedlings below nurse shrubs are less likely to be eaten or damaged than in bare patches (Aerts et al. 2007). Further nurse shrubs can improve the mycorrhizal soil infectivity and microbial soil functionalities (Williams 2012). The presence of nurse shrubs can also increase nutrient levels and alter pH levels (Abiyu 2012). Plants have different access to nutrient pools because of different rooting depth and growing season (Whisenant 2001) and nutrient transfer between species can occur through mycorrhizal connections (Chiarrello et al. 1982). If one species mines different resources than another and transfers those otherwise unavailable nutrients to another species, not just reduced competition, but facilitation is possible. Further, as some species are able to access nutrients, which are unavailable to another, those nutrients can become available after cycling through the first plant (Vandermeer 1989).

In systems where a critical source has a flow rate through the system, certain species might act to reduce this flow rate of the resource out of the system, which might benefit other species (Perfecto et al. 1986). ‘This is particularly relevant to nitrogen, since its rate of movement through soils can be rapid’ (Whisenant 2001). But if nitrogen is transferred, what exactly is the mechanism of transfer and how can we investigate it? This question will be discussed in later chapters.

### 2.2.2. Limits of nurse shrubs

However facilitative, nurse shrubs are at the same time competitors for water, light, (Aerts et al. 2007; Boehm 2011; Høgh-Jensen, Schjoerring 2000) and nutrients. Especially at low levels of stress the facilitative effect might be overruled by competitive impacts while at extreme stress the amelioration is insufficient to facilitate growth or survival. Therefore the
facilitative effect of nurse shrubs might be highest under moderate conditions and may disappear under most stressful conditions (Fig. 6) (Holmgren, Scheffer 2010). On the other hand facilitation might be highest under stressful conditions because, depending on the respective coping strategies, the stress might impair the abilities to compete. Therefore the interaction can also shift from competition towards facilitation as the environment becomes more stressful (Maestre et al. 2009).

The facilitation might not only shift along the stress gradient but also along the development stage of the beneficiary (Alemayehu 2007).

This implies that nurse shrubs may expand the range of conditions where organisms may occur, but do not necessarily boost their performance (Holmgren, Scheffer 2010).

![Fig. 6 Schematic representation of the role of facilitative amelioration of water stress in plant communities (adapted from Holmgren, Scheffer 2010).](image)

### 2.3. Limits of restoration

Invasions or extinctions caused by human activities have altered ecosystem goods and services and many of those changes are difficult, expensive or impossible to reverse (Whisenant 2001; Hooper et al. 2005; Chazdon 2008). Even after many decades regenerating forests are different from their original conditions (Chazdon 2008). Particularly tropical systems are difficult to restore and it is unlikely, that the numerous species present before the disturbance will all recolonize (Holl et al. 2000).

Therefore reference systems for ecological restoration might change over time. Irreversible environmental conditions, like severe soil erosion, can prevent a return to the past. This makes it necessary to consider other options or, in other words, develop a series of successive reference states or systems (van Andel, Aronson 2012).
3. **Nitrogen isotopes**

As discussed in previous chapters, to restore an ecosystem it is important to first remove barriers which prevent restoration. Impaired nitrogen supply is one possible barrier. Nitrogen is considered as limiting factor for plant growth, therefore it is essential to understand the nitrogen fluxes within a system and restore pre-degradation nitrogen sources or provide nitrogen through other sources.

Nitrogen has two stable isotopes: \(^{14}\)N, which is prevalent and \(^{15}\)N, which varies due to fractionation in biological processes. Atmospheric N\(_2\) has a \(^{15}\)N abundance of 0.3663 atom\%. “This natural abundance is commonly expressed in \(\delta\) units, which denote parts per thousand deviations ( ‰) from the ratio of \(^{15}\)N : \(^{14}\)N in atmospheric N\(_2\) (Junk, Svec 1958; Högberg 1997). Within a system there can be different pools or compartments of nitrogen with distinctly different \(^{15}\)N abundances (**Fig. 7**). Nitrogen from those sources has an isotopic signature different from the mean value of the system. Therefore variations in stable nitrogen isotope ratios could provide information about sources of nitrogen used by plants and fluxes of nitrogen in ecosystems.

![Fig. 7 Samples of surveys attempting to identify N\(_2\) - fixing spp. (●) in mixtures with non - N\(_2\) - fixing spp. (○) based on differences in \(^{15}\)N natural abundance. Data from (b) a miombo woodland in Zambia and (c) a lowland rainforest in Cameroon (adapted from Högberg, Alexander 1995)](image)

3.1. **Factors determining \(\delta^{15}\)N ratios**

Variations of isotope ratios result from equilibrium and kinetic isotope effects. Larger activation energy is required to dissociate an isotopically heavy chemical species than a light one and heavier molecules or ions react more slowly than isotopically lighter compounds.
These processes, also called fractionation, are described by enrichment: the product relative to that of the substrate, which is expressed in per mil (‰). Another term for it is discrimination (Δ). The fractionation rate depends on the specific reaction and the reaction sequence, since the fractionation during the rate-limiting step determines the net overall fractionation for the entire reaction sequence (Högberg 1997). Nitrogen fractionation in soil plant systems is most likely to occur at ammonia volatilization, nitrification, uptake by symbiotic fungi and uptake by plants (Högberg 1997).

Variations in substrate supply, abiotic conditions and composition of organism assemblages, (such as the type of mycorrhiza, bacterial symbiont and plant host) and their demand for nitrogen dynamically affect and change the isotopic signatures of the various nitrogen species (Abuzinadah et al. 1986; Legard 1989). For example, if the nitrification is rapid and leaves little NH₄⁺ (which is usually enriched) behind, plants will shift to NO₃⁻ as nitrogen source and as a result plant δ₁⁵N should become lower.

Plants are integrators of δ₁⁵N in available nitrogen sources, but their signature might be affected by the signature of plant stored nitrogen in addition to the signature of recent soil-derived nitrogen.

Contrary to biologically active pools, which might change over short time periods, the δ¹⁵N of soil total nitrogen is dominated by the isotopic signature of stable nitrogen, which is not likely to change over decades (Johannisson, Högberg 1994). The isotopic signature of a nitrogen source is not constant, but depends on its origin and the character of nitrogen transformations in the specific system. Therefore data on δ¹⁵N cannot be used directly in comparisons between ecosystems, but for interpretations of plant nitrogen sources within ecosystems (Högberg 1997).

To sum it up, the δ¹⁵N abundance of plants depends on (1) the source of plant nitrogen (e.g. soil, precipitation, gaseous nitrogen compounds, N₂-fixation), (2) the depth in soil from which nitrogen is taken up, (3) the form of nitrogen used (e.g.NH₄⁺,NO₃⁻, organic nitrogen sources), (4) the influence of mycorrhizal symbioses and fractionations during and after nitrogen uptake, (5) plant phenology and (6) interactions between those factors (Högberg 1997; Nadelhoffer et al. 1996).

### 3.2. Investigating δ¹⁵N

Biological variation of nitrogen isotope ratios between, e.g. individuals of the same plant species is usually larger than the analytical error and if isotopic pairs of more than one element are analyzed, one can distinguish the isotopic source effect from the effect of isotope
fractionation. This means that a meaningful interpretation of source effects could be possible (Högberg 1997; Høgh-Jensen, Schjoerring 2000). For example a significant change of δ\(^{15}\)N of a non-N\(_2\)-fixing species next to a N\(_2\)-fixing species could indicate a transfer of fixed nitrogen from one species to another (van Kessel et al. 1994; Hoogmoed et al. 2014).

However, the δ\(^{15}\)N of nitrogen deposition varies considerably spatially and temporally and also the δ\(^{15}\)N of available soil nitrogen has a large variability. For example differences in rooting patterns and variation in δ\(^{15}\)N with soil depth can influence the isotopic signature of plant nitrogen (Ledgard et al. 1984; Högberg 1997). Accordingly it is difficult to assess contributions from different soil nitrogen sources solely based on δ\(^{15}\)N. Therefore additional independent non-isotopic or δ\(^{15}\)N tracer data or δ\(^{15}\)N modeling should be used to interpret the contributions of different nitrogen sources to plant δ\(^{15}\)N and target and reference species should only be sampled when in close proximity (Shearer, Kohl 1986).

The natural abundance method (Amarger et al. 1977; Shearer, Kohl 1986) compares the δ\(^{15}\)N of a N\(_2\)-fixing species (N\(_{\text{fix}}\)), with a non-N\(_2\)fixing reference species (N\(_{\text{ref}}\)), which rely solely on soil derived nitrogen.

The nitrogen derived from N\(_2\)-fixation (N\(_{\text{dfa}}\)) can be calculated as follows (Amarger et al. 1977):

\[
N_{\text{dfa}} = (\delta^{15}N_{\text{ref}} - \delta^{15}N_{\text{fix}})/(\delta^{15}N_{\text{ref}} - B)
\]

B is a reference value and describes the δ\(^{15}\)N of the N\(_2\)-fixing plant when totally dependent on N\(_2\). It accounts for the fractionation during the process of fixation. B can vary with bacterial strain and abiotic factors. Reference species may have positive or negative δ\(^{15}\)N, the equation will work either way (Vitousek et al. 1989).

The advantage of this method is that nothing has to be added or disturbed. Thus it can be used in field research, where other methods are not feasible, e.g. when investigating nitrogen fixation of deep-rooted plants. The disadvantage is that the compared species, in order to be comparable, must have (1) similar root distributions, (2) similar temporal nitrogen uptake patterns and (3) the same preferences for the various species of inorganic and organic nitrogen in the soil (Högberg 1997).

In systems where complementary data are lacking a difference in δ\(^{15}\)N of at least +5 or -5‰ in δ\(^{15}\)N between non- N\(_2\)-fixing reference species and nitrogen derived by N\(_2\)-fixation (B) is necessary. Further there should not be any large unexplained variability in δ\(^{15}\)N amongst reference species (Högberg 1997).
If the difference in δ^{15}N between non- N\textsubscript{2}-fixing species and B is too small, one cannot be certain about the quality of the reference species meets the requirements 1-3. Therefore the method can only provide an indication whether a species is N\textsubscript{2}-fixing or not. In lesser known types of vegetation this information can be very valuable (Högberg 1997).

3.3. \textsuperscript{15}N spatial variation in soils

The spatial variation of \textsuperscript{15}N in undisturbed forest soil profiles can provide information about the N-cycle in forest ecosystems: comparatively low \textsuperscript{15}N abundance in the surface layer indicates nitrogen limitation and low rates of nitrification, whereas high \textsuperscript{15}N abundance in the surface layer compared to deeper layers indicates high rates of nitrification (Högberg P. et al. 1996; Näsholm et al. 1997; Nohrstedt et al. 1996).

δ\textsuperscript{15}N is lower in soil surfaces than further down in the soil, due to redeposition of \textsuperscript{15}N depleted plant N onto the soil surface by litter-fall (Nadelhoffer, Fry 1988, 1994). In forest soils the increase in the upper dm of soil can be between 5 and 10‰ or higher (Högberg P. et al. 1996; Piccolo et al. 1996). The major cause of this is probably fractionation against \textsuperscript{15}N during the mineralization-plant uptake pathway and deposition of this \textsuperscript{15}N -depleted nitrogen onto the soil. This pattern can be disrupted by human activity or activity of other animals, mixing the soil layers (Högberg 1997).

Ammonia volatilization from litter during decomposition, tends to enrich the remaining nitrogen with \textsuperscript{15}N (Turner et al. 1983). Another process to consider is the possibility of in situ synthesis of \textsuperscript{15}N-enriched compounds by microbes during decomposition. These compounds, if recalcitrant, accumulate with increasing soil depth, which might lead to an increase in δ\textsuperscript{15}N down the profile.

Disturbances, like fire, might not only consume the upper δ\textsuperscript{15}N-depleted surface layer but also force plants to find nitrogen in lower horizons. This can lead to an increase of δ\textsuperscript{15}N in plants, caused by and increased nitrification after the fire (Raison 1979) by providing \textsuperscript{15}N-enriched NH\textsubscript{4}+ (Högberg 1997).

Another factor influencing δ\textsuperscript{15}N is the availability of nitrogen and the preferred nitrogen species of the surrounding vegetation. Limited availability of nitrogen and thus lower nitrification means no isotopic enrichment of NH\textsubscript{4}+. If NH\textsubscript{4}- is preferred by the vegetation, uptake can lead to depleted nitrogen at the soil surface, as isotopically depleted litter is mineralized and taken up again. On the contrary, in situations where high nitrogen inputs promote nitrification, and thus δ\textsuperscript{15}N-enriched NH\textsubscript{4}+, while NH\textsubscript{4} – nitrogen is preferentially
used as nitrogen source, the surface soil will progressively enrich (Högberg P. et al. 1996; Högberg 1997).

3.4. **$^{15}$N variation in plants**

Metabolic processes within plants can lead to variations in $\delta^{15}$N between different plant parts, which are large enough to interfere with the interpretation of N source effects. This applies especially to fruits and senescent parts.

The $^{15}$N abundance in plants can vary seasonally, among individuals, leaf ages, canopy position of leaves, nitrogen abundance in soil (Högberg 1997; Högberg P. et al. 1996; Gebauer, Schulze 1991; Näsholm 1994; Kalcsits et al. 2015).

$^{15}$N in N$_2$-fixing plants can show positive and negative discrimination factors. The $\delta^{15}$N of N$_2$-fixing plants can vary in nodule and shoot and depends on the identity of the micro symbiont. Regarding the whole N$_2$-fixing plant there is no evidence for isotope discrimination associated with legume symbiotic N$_2$ fixation per se (Unkovich 2013).

Therefore, as stressed above, data on $^{15}$N can provide valuable insights into unknown systems but it is difficult to assess contributions from different soil nitrogen sources solely based on $\delta^{15}$N. Additional independent non-isotopic or $\delta^{15}$N tracer data or $\delta^{15}$N modeling should be used to interpret the contributions of different nitrogen sources to plant $\delta^{15}$N and target and reference species should only be sampled when in close proximity.

3.5. **Implications of $^{15}$N abundance for ecosystem restoration**

Establishing a physical environment that sustains reproducing populations of species is a key feature of successfully restored ecosystems (Society for Ecological Restoration International 2004). Therefore assessing and removing abiotic barriers hindering unassisted restoration, like limited nitrogen supply, is vital to start a restoration process (Whisenant 2001).

As argued above, $^{15}$N abundance, if combined with other data, provides insights into the nitrogen balance of an ecosystem and can provide a starting point for assessing those barriers and potential solutions related to nutrient supply of target species.

Further information about $^{15}$N abundance in forest soils can be used to provide ecosystem services by aiding the development sustainable forest management plans (Eshetu 2004).
4. **Mycorrhiza and ecosystem restoration**

Ecosystem recovery requires the reestablishment and stabilization of energy sources that drive belowground processes (Perry et al. 1989). Plants provide the energy that fuels these biological processes (Whisenant 2001). Even though there are resting stages of many rhizosphere organisms, many of them are consumed by saprophytes and erosion losses. Therefore soil organisms, like mycorrhiza, may require certain plant species or guilds for their continued existence and might not be able to adapt to the disappearance of plants. Reduced energy input from plants also affects the physical characteristics of soil, which can lead to the decline of other soil organisms (Whisenant 2001).

4.1. **Function of mycorrhiza**

Mycorrhizal colonization has positive effects on stomatal conductance, photosynthetic rates, water use efficiency, nutrition and growth across species (Querejeta et al. 2003; Seifi et al. 2014) by improving the hosts uptake of nitrogen, phosphorous and all other essential plant nutrients. Mycorrhizae can also improve the stress resistance (Meddad-Hamza et al. 2010). Therefore mycorrhizae directly improve the growth of their hosts (Seifi et al. 2014). Furthermore nutrients can be transferred from one species to another through mycorrhizal connections (Chiariello et al. 1982). If one species can access different sources than another, otherwise unavailable nutrients can be transferred, which makes mycorrhiza facilitators (Vandermeer 1989).

Mycorrhiza are also important drivers for the recovery of soil aggregate stability in post disturbance communities (Duchicela et al. 2013; Miller 1987). The introduction of shrubs can improve the mycorrhizal community either directly, by introducing mycotrophic shrubs or indirectly and through attracting other mycorrhizal plants and providing microsites (Miller 1987; Williams 2012).

4.2. **Evaluating the status of mycorrhiza**

Mycorrhization can be examined either by looking at (1) the root system with external hyphae and spores, (2) the spores when separated from the soil, (3) the wall structure of the spore or (4) the cleared and stained roots. Because vesicular arbuscular mycorrhiza is characterized by the presence of arbuscules one way to analyze it is to examine cleared and stained roots. This can be difficult since these structures can be absent from field-collected roots for various reasons (Brundrett 1996):
- Roots of many species persist in the soil for months or years without secondary growth, but arbuscules last only for a few weeks (Brundrett, Kendrick 1990).
- Roots from the field are often heavily pigmented and may require a post-clearing bleaching step. The bleaching reduces the staining of fungal structures and the delicate branch hyphae of arbuscules will be the first structures to be affected.
- Root anatomy can restrict the arbuscules to a single cortex layer (Brundrett, Kendrick 1988).

To avoid this, young roots with growing tips should be collected during seasons favoring mycorrhizal activity (temperature and moisture).

Due to the mentioned problems with the presence of arbuscules hyphal colonization alone is often used to identify VAM associations. However, VAM fungi also occupy non-host roots. Therefore confirming an association requires prior knowledge about root phenology, and prior observations of that plant species (Brundrett 1996).

Plants within a genus usually have the same type of mycorrhizas and these relationships are generally consistent within a family (Harley, Harley 1987; Brundrett, Abbott 1991). However it is difficult to safely predict the type of mycorrhizal associations or their functional significance of a given plant species (Brundrett 1996).

Species either have (1) consistently high levels of mycorrhizas, (2) intermediate or variable levels of mycorrhizas, or (3) are not mycorrhizal (Brundrett, Kendrick 1988). According to those variations plants can be classified into obligatory mycorrhizal, facultatively mycorrhizal and non-mycorrhizal species which display typical features (Table 1) (Brundrett 1996).

Table 1 Typical features of host root systems and mycorrhizal formation that are associated with categories of mycorrhizal formation (Brundrett 1996)

<table>
<thead>
<tr>
<th>Designation</th>
<th>Obligate</th>
<th>Facultative</th>
<th>Non-mycorrhizal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colonisation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arbuscules</td>
<td>young roots</td>
<td>sparse or variable</td>
<td>none</td>
</tr>
<tr>
<td>Hyphae or vesicles</td>
<td>older roots</td>
<td>sparse or variable</td>
<td>may occur in old roots</td>
</tr>
</tbody>
</table>
Plants with facultatively mycorrhizal associations are more common in habitats with limited plant productivity because of soil conditions, like very dry, wet or cold habitats as well as disturbed habitats with limited mycorrhizal inoculum (Brundrett 1991).

4.3. Glomalean fungi
Research on *Olea europaea subsp. sylvestris* showed that inoculation with *Glomus intraradices* significantly enhanced colonization with arbuscular mycorrhiza, growth and nutrition of seedlings. Inoculated plants had a 50% higher photosynthetic rate, while at the same time exhibiting higher water use efficiency. The plants showed greater responsiveness to *Glomus intraradices* infection during a wet period (Querejeta et al. 2003).

4.4. Mycorrhiza and nitrogen
Certain types of mycorrhiza play a vital role in N uptake and probably exert a major control on plant $\delta^{15}$N (Michelsen et al. 1996; Michelsen et al. 1998). However interpretations of $\delta^{15}$N patterns are only correct, if there is no fungal discrimination against $^{15}$N during the uptake or assimilation, as well as during mycorrhizal transfer of nitrogen to vegetation (Aerts 2002; Hobbie et al. 1999).

4.5. Implications of mycorrhiza for ecosystem restoration
The removal of plant species can set off a feedback loop through which the soil community is changed, which in turn influences the growth rates of the involved plant species. Those interactions are not homogenous: soil organisms respond differently to host identity and individual soil organisms affect plant growth differently. The resulting positive and negative feedback in soil communities can be vital for rejuvenation of existing species or the establishment of new species (Bever 2003).
Disturbances such as agricultural land use, flooding, drought and erosion or grazing can alter mycorrhizal communities, which might result in the elimination or reduction of mycorrhizal fungi (Duchicela et al. 2013; Powell 1980; Grigera, Oesterheld 2004). Mycorrhizae are frequently absent during the early stages of succession because many plants characteristic of these stages are either non-mycorrhizal or facultatively mycorrhizal (Reeves et al. 1979). This precipitates a progressive decline in the number of viable mycorrhizal propagules within the soil. If the native plant community is composed mainly of obligatory mycorrhizal plants, changes in in the mycorrhizal community cannot simply be reversed (Miller 1987).
If mycorrhizal propagules are absent they require reintroduction (Miller 1987). However, this is not an easy task as “Easily decomposed organic materials, fertilization and irrigation reduce infection rates of mycorrhizal fungi and rhizobia” (Whitford 1988).

5. Ethiopia – facts and figures

The Federal Democratic Republic of Ethiopia is located close to the equator in eastern Africa and consists of nine ethnically based states. The capital, Addis Ababa, is located at the center (Fig. 8). Ethiopia’s land area is approximately 1,000,000 km² and its elevation varies between -125 and 4,533m. Due to those this diverse topography Ethiopia has 18 major and 49 minor agro-ecological zones (Ethiopian Biodiversity Institute 2014). Most of the area is characterized by Dry Kolla (dry lowlands), Dry Bereha (dry hot-lowlands) and Moist Kolla (moist lowlands) (Mundy 2005). The climate is marked by tropical monsoon climate which leads to regularly occurring dry and rainy periods. Current ecological issues are deforestation, climate change, desertification and water shortages (Central Intelligence Agency 2015).

Fig. 8 Location and administrative districts of the Federal Democratic Republic of Ethiopia. The study was conducted in the Amhara Region (light green) (adapted from Ethiopia Districts - Ethiopia maps,).

5.1. Population and poverty

The population is estimated at 96 to 99 million inhabitants and is composed of about fourteen ethnic groups, the biggest of them are the Oromo, The Amhara, the Somali and the Tigray. 43, 90% of the population is between 0 and 14 years old and the life expectancy at birth is 64 years. The average birthrate is 5, 15 children per woman, which is also reflected by the growth rate of 2,89%. The population below the national poverty line is estimated at around 35% (2012 est.) (Central Intelligence Agency 2015; The World Bank Group 2015). The Multidimensional Poverty Index is estimated at 0,564, which means that 87, 3% of the population is deprived at least on third in one of the three factors education, health and
standards of living. The most deprived asset is the living standards, which are composed of electricity, sanitation, drinking water, floor, cooking fuel and assets. In Ethiopia it accounts for almost half of the deprivation. Further there is a high disparity between urban (MPI 0.230) and rural areas (MPI 0.637), which means that rural areas are more deprived, poorer, than urban areas (Fig. 9) (Oxford Poverty and Human Development Initiative 2015).
Fig. 9 Disparity between urban or rural areas and the contribution of different factors to poverty of urban or rural areas in Ethiopia (Oxford Poverty and Human Development Initiative (OPHI) 2015)
5.2. Economy
The GDP (official exchange rate) is estimate between 52.34 and 54.80 billion USD (2014 est.) (The World Bank Group 2015; Central Intelligence Agency 2015). Most of it is composed by agriculture (47, 7%) and services (41, 9%). Even though services make up more than one third of the GDP most inhabitants are occupied with agriculture (85%). Information about land use varies, but range around 36% agricultural land, 12, 2% forest land, and 51, 5% other land. The biggest sectors for agriculture are arable land/annual crops (15,2%) and permanent pasture (20%)(2011 est.) (Central Intelligence Agency 2015).
Ecosystem services contribute an estimated 4% to the GDP by providing honey, forest coffee, natural gums and timber and the economic value derived from protected areas is estimated at 1, 5 billion USD per year (Ethiopian Biodiversity Institute 2014)
19,5% of the population is living in urban areas, the rest of the population are mainly subsistence farmers (Central Intelligence Agency 2015), producing mainly maize, sorghum, teff, wheat, barley and cattle (Central Statistical Agency 2014a).
More than 80% of those farmers cultivate areas smaller than 2, 1 hectares, which total 52, 3 % of the crop area. Almost 50% of the farmers supply for households with more than three members (Central Statistical Agency 2012).
The most common methods to protect agricultural land from erosion are terracing, ploughing along the contour, water catchments and planting trees. Planting trees is by far the least popular method to prevent erosion (Central Statistical Agency 2014a). Land use data shows, that only 1,47% of the land use type is wood land and most of the holdings within this land use type are smaller than 5,01 hectares (Central Statistical Agency 2014b).

5.3. Energy
In 2012 only 26, 6% of Ethiopians had access to electricity (The World Bank Group 2015). Among wood, dung, crop residues, kerosene and charcoal, wood and dung are the main fuel sources for rural households (Gebreegziabher 2007). The fuel wood consumption by households in 1995 was estimated at 64.903m³ and has risen to 79.445m³ in 2012 (UNdata 2015). Dung and wood are mainly self-collected and when wood is scarce dung becomes the dominant source, which has negative implications for the use of dung for improving soil fertility (Gebreegziabher 2007; Bekele, Mekonnen 2013). The next most important source is kerosene, which is always bought (Gebreegziabher 2007).
5.4. Biodiversity in Ethiopia

As shown in Table 2, Ethiopia is rich in biodiversity. There are 20 national parks, three wildlife sanctuaries, two wildlife reserves, 17 controlled hunting areas, seven open hunting areas, three community conservation areas (Ethiopian Wildlife Conservation Authority,) and 58 national forest priority areas of which 37 are protected (Million, Leykun 2001).

Table 2 Species of Ethiopia (adapted from Groombridge et al. 1994)

<table>
<thead>
<tr>
<th></th>
<th>Total number of species</th>
<th>Endemic Species</th>
<th>Threatened Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mammals</td>
<td>255</td>
<td>31</td>
<td>21</td>
</tr>
<tr>
<td>Birds</td>
<td>813</td>
<td>29</td>
<td>17</td>
</tr>
<tr>
<td>Reptiles</td>
<td>-</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Amphibians</td>
<td>-</td>
<td>30</td>
<td>-</td>
</tr>
<tr>
<td>Plants</td>
<td>6500</td>
<td>1000</td>
<td>153</td>
</tr>
</tbody>
</table>

Main direct threats to Ethiopia’s biodiversity are habitat conversion, unsustainable utilization of biodiversity resources, invasive species, replacement of local varieties and breeds, climate change and pollution. Especially overgrazing and browsing by livestock has contributed to the degradation of rangelands and forest ecosystems. Indirect threats are demographic change, poverty and lack of alternative energy resources (Ethiopian Biodiversity Institute 2014; Ethiopia, Ministry of Finance and Economic Development, Ethiopia, United Nations Country Team 2012).

5.5. Rehabilitation and restoration

Ethiopia’s early Juniperus-Podocarpus forests were cleared about 500 BC and were replaced by scrub and grassland vegetation, which was maintained by grazing of domestic stock. Dry conditions during the ‘Medieval Warm Period’ combined with anthropogenic pressure caused land degradation. Increased rainfall during the sixteenth to eighteenth centuries led to a regeneration of Juniperus forests, which was followed by another period of deforestation (Darbyshire et al. 2003). Especially during the late-feudal period and the civil war, vegetation cover was at a minimum (Lanckriet et al. 2015).

The ministry of agriculture is conducting restoration and rehabilitation measures, among them area closure (exclosures), integrated community based watershed management and natural forest management.
Two main types of area closures are implemented (a) closing area from livestock and people for natural regeneration and (b) closing area from livestock and people for assisted regeneration with planting seedlings, mulching and establishing water harvesting structures to speed up the process (Ethiopian Biodiversity Institute 2014).

Soil and water conservation structures supported with biological conservation measures planting of multipurpose trees, shrubs and grasses have been carried out in 57,000 community based watersheds which cover about 13 million hectares of land. Forest management plans have been prepared for 1.4 million hectares of natural forests and about 2.9 million hectares of land have been afforested with indigenous and exotic tree species (Ethiopian Biodiversity Institute 2014).

Thanks to these approaches the rehabilitated area increased from 3.2 million hectares in 2009 to 7 million hectares in 2013 (Ethiopian Biodiversity Institute 2014). This success is underlined by recent rises in pollen of Dodonea and pioneer (Lanckriet et al. 2015).

5.6. Climate

The climate is marked by tropical monsoon climate which leads to seasonal dry and rainy periods. Most regions experience one main wet season from mid-June to mid-September, while parts of northern and central Ethiopia have a secondary wet season from February to May (Fig. 10). The southern regions experience two distinct wet seasons from March to May and from October to December. The durations and the onset of the rainfall seasons vary considerably interannually, causing frequent drought (Central Intelligence Agency 2015; McSweeney et al.,). The average monthly temperature and rainfall from 1900 to 2012 shows rainfall variations between 7.1mm in January and 132.1mm in August. The mean temperature varies between 21.2°C in December and 24.2°C in April (Climatic Research Unit of University of East Anglia,).

Fig. 10 Climate of Ethiopia (The World Bank Group 2015)
The mean annual temperature has increased by 1.3°C between 1960 and 2006 and daily temperature observations show significantly increasing trends in the frequency of hot days and hot nights, while the frequency of cold days has decreased. Projections of future climate estimate a temperature increase by 1.1°C to 3.1°C and indicate an increase in the frequency of days and nights that are considered “hot” in current climate (McSweeney et al.).

Projections for precipitation indicate increases in annual rainfall due to increasing rainfall in the “short” rainfall season in southern Ethiopia, while slight decreases in the north east are expected. Models also indicate increases in the magnitude of 1- and 5-day maxima from 0 to +29mm and -4 to +40 respectively (McSweeney et al.).
6. **Olea europaea subsp. cuspidata**

*(O. cuspidata, O. europaea subsp. africana, O. africana)*

Vernacular names: Wild Olive, Brown Olive

Family: Oleaceae

*Olea europaea subsp. cuspidata* is a slow growing, indigenous tree or shrub, growing (5) 10 – 15m high. It has a multi – branched, rounded crown with grey-green, evergreen foliage (**Fig. 11, Fig. 12**).

![Fig. 11. Grey-green, evergreen leaves of *Olea europaea subsp. cuspidata* growing at the exclosure site at Ambo Ber (own work)](image1)

![Fig. 12. Habit of *Olea europaea subsp. cuspidata* (Forest and Kim Starr, 2009)](image2)

**6.1. Distribution range**

*Olea europaea subsp. cuspidata* occurs in almost all floristic regions of Ethiopia and in six of Ethiopia’s agro-ecological zones: Moist Mid-highland, Wet Mid-highland, Dry Highland, Moist Highland, Wet Highland and Dry Frost area. It grows best in Moist and Wet Mid-highlands and lower Mid-highlands. It further occurs throughout tropical East Africa to South Africa, Arabia, the Himalayas, and Southwest Asia (Hedberg 2003; Bekele-Tesemma, Tengnäss 2007).

**6.2. Ecology**

The *Olea europaea subsp. cuspidata* is native to evergreen Juniperus – Podocarpus forests (Bekele-Tesemma, Tengnäss 2007; Hedberg 2003). It is widely distributed in dry forest and forest margins at altitudes between (750) 1700 – 2700 (3000)m (Dharani 2011) (Hedberg...
Olea europaea subsp. cuspidata has, like other typical Ethiopian highland species, a high seed predation, but also low seed viability and low germination rate. 18 months after burial in a forest 73% of seeds were dead, 20% were still intact, while only 7% where germinated (Wassie et al. 2009).

Olea europaea subsp. cuspidata germinates in forested areas, gaps as well as in completely open areas. Highest germination rates occur under light conditions. Also the seedling survival is higher in gaps than under canopy shade. Growth however is equally well in under closed canopy and gaps, while it is retarded in completely open area (Alemayehu 2007).

Seedlings had a higher survival rate when planted during the summer rains instead of spring rains. They probably do not require high rainfall intensities but an extended rainy season for survival (Aerts et al. 2006b).

6.3. Propagation

Olea europaea subsp. cuspidata is a poor seeder, with about 8000 seeds per kg, which can be stored for about two months. The germination is low and slowly (45 – 90 days) (Wassie et al. 2009; Bekele-Tesemma, Tengnäs 2007; Piotto, Di Noi 2001). Pre-seeding treatments for fresh seeds are not obligatory for germination, but they can increase germination rate and speed (Bekele-Tesemma, Tengnäs 2007; Piotto, Di Noi 2001). Old seeds should be soaked in water for 48 hours (Bekele-Tesemma, Tengnäs 2007). Other possible treatments are chemical scarification with (a) sulfuric acid (5-10 minutes) or (b) soaking in a 3% solution of Na2CO3 for five hours, followed by immersion in a 0.5% solution of KOH for six hours. Both of those treatments boosted the germination to over 70%. Other experiments with related species of the genus Olea suggest that seeds germinate rapidly when the woody endocarp is removed (Piotto, Di Noi 2001).

6.4. Ecological importance

Olea europaea subsp. cuspidata is an essential species in the Ethiopian Highlands. Research suggests that about a third of Ethiopia’s now agricultural land was previously covered by broadleaved evergreen and deciduous forest, as well as closed to open shrubland and mosaic forest-shrubland/grassland (Hailu et al. 2015). These forests probably consisted mainly of Juniperus sp. and Podocarpus sp. Other woody species within those forests were Olea,
Hagenia, Euclea, Acacia, Myrica, Dodonea, Clematis, Galiniera, Celtis, Pittosporum and Acacia (Darbyshire et al. 2003; Lanckriet et al. 2015).

Furthermore Olea europaea subsp. cuspidata is able to store more carbon than other species and has a high potential for contributing to aboveground C-stocks (Mokria et al. 2015). Olea europaea subsp. cuspidata forms arbuscular mycorrhiza, which is predominant in the dry afro-montane forests of Ethiopia (Wubet et al. 2009) and could therefore provide a stepping stone for mycorrhizal recolonization (Miller 1987; Reeves et al. 1979; Williams 2012).

6.5. Socio-economic importance

The seven most important criteria for Ethiopian farmers for tree species selection are the provision of construction wood, (agricultural) tools, food, firewood and charcoal, fodder, growth speed and drought resistance (Reubens et al. 2011). Olea europaea subsp. cuspidata addresses most of those criteria (Dharani 2011; Bekele-Tesemma, Tengnäs 2007; Reubens et al. 2011), is well known and considered most important by local stakeholders (Reubens et al. 2011).

Furthermore Olea europaea subsp. cuspidata has a wide range of other uses such as medicine against tapworm or itchy rashes, fumigation for traditional drinks and milk or as toothbrushes (Hedberg 2003; Bekele-Tesemma, Tengnäs 2007). Its wood is used to produce quality furniture and woodcarvings, for poles, posts, paneling, flooring, walking sticks, general timber, firewood and charcoal (Dharani 2011; Bekele-Tesemma, Tengnäs 2007). The fruits are edible and sometimes used for oil (Hedberg 2003).

The tree can further serve as bee forage and as a windbreak (Hedberg 2003; Bekele-Tesemma, Tengnäs 2007; Dharani 2011).

Due to the historic distribution of Olea europaea subsp. cuspidata in the Ethiopian Highlands, its adaption to dry climatic conditions and its wide range of uses the restoration of Olea europaea subsp. cuspidata would not only contribute to biodiversity conservation but also improve the livelihoods of locals on many levels.
7. **Materials and methods**

7.1. **Study area**

The study was conducted at an exclosure of six hectares (Fig. 14, Fig. 15) which is within the area of the village Ambober. The village is located north of Lake Tana in North Gonder, which is a part of the Amhara Regional State in northwestern Ethiopia (Fig. 13). The area is characterized by Moist Weyna Dega (Moist Mid-Highlands) and Moist Dega (Moist Highlands) (Mundy 2005).

![Location of study area](image)

Fig. 13 Location of study area (dark green) within the administrative district Amhara (light green) and the North Gonder Zone (medium green), as well as the location of the weather stations Maksegnit and Addis Zemen (adapted from Ethiopia Districts - Ethiopia maps).

7.2. **Land use**

The village is connected by 10km dry weather road to the main highway that connects the regional capitals and the country with neighboring nations in the west. The geographic location connects the area to a growing national and regional market. The average land holding is 0.56 hectares. The farming system is a mixed crop livestock system where trees form valuable components. The area is a transition zone between low production potential cereal-livestock zone in the east and high production potential cereal-livestock zone in the west and south. A typical household is entitled to three parcels of land. The first parcel of land
is around the homestead, the second and the third parcels are away from the homestead (Abiyu 2012).

The exclosure was established through a participative process with the local farming community in 2009 on degraded, open access grazing land. It already contained naturally regenerated shrubs of the following species: *Dodonea angustifolia*, *Croton macrostachys*, *Vernonia sp.*, *Maytenus senegalensis*, *Rhus glutinosa*, and *Otostegia integrifolia*. To test for the effects of artificially established nurse shrubs the species *Senna didymobotrya* and *Ficus thonningii* were introduced into the exclosure (Abiyu 2012).

According to the negotiated rules guards are elected and assigned to monitor the exclosure, communicate the purposes, to plant high quality fodder trees and to pass sanctions for the violation of the rules like ruminants inside the exclosure (Abiyu 2012).

This area was selected because of the establishment of an exclosure in 2009, which is now, five years later, ideal for researching the natural regeneration of *Olea europaea subsp. cuspidata*. Due to the constricted and insecure land use establishing a new exclosure would have exceeded the scope of the study.

Geologically the study area is situated in the Tarmaber Gussa Formation, which was formed between the Oligocene and the Miocene and is constituted by alkaline to transitional basalts. It often forms shield volcanoes with minor trachyte and phonolite flows (Teshome, Woldie 1996). The Formation is part of the northwestern shoulder of the Ethiopian plateau, which has
a higher crustal thickness than the southwestern part and it is characterized by east-west trending transverse lineaments (Alemu 2012).

The surrounding ecosystems can be described as montane grassland and dry evergreen montane forest and evergreen shrubs. Montane grasslands can be found between 1,500 and 3,200m and are characterized by trees and shrubs, interspersed with grasses. They occur in areas where intense cultivation and livestock husbandry have led to considerable land degradation. Evergreen montane forest and evergreen shrubs can be found between 1,500 and 3,200m. *Olea europaea subsp. cuspidata* is typical for both of these ecosystems (Ethiopian Biodiversity Institute 2014).

Research suggests that before transformation into agricultural land most of the area was covered by mosaic forest-scrubland and closed to open scrubland (Hailu et al. 2015).

Personal recounts from villagers claim that more than twenty years ago the whole area was covered in forest.

7.3. Soil

The soil type at the exclosure site varies between leptosols and acrisols. The pH-values between 0 and 50cm vary between 6, 5 and 6, 8. The bulk density varies between 1, 07 and 1, 52 (Abiyu 2012).

7.4. Climate

The area is characterized by tropical monsoon climate with a distinct dry period from September to June (Fig. 16, Fig. 17) (Klima: Maksegnit,; Klima: Adis Zemen,). The average temperature is 20, 2°C. The minimum is around 11, 3°C and the maximum is around 30, 2°C. The temperatures fluctuate around 4°C within the year. The minimum precipitation is 2mm in January, the maximum precipitation is 317mm in July (Klima: Adis Zemen,; Klima: Maksegnit,).
Fig. 16 Climate at Adis Zemen (Klima: Adis Zemen,)
7.5. Sampling
To find out whether there are nurse shrubs or not and which of the occurring species would be most likely nurse shrubs for *Olea europaea subsp. cuspidata* we defined two settings: associated and not-associated. In the associated setting *Olea europaea subsp. cuspidata* is surrounded by other shrubs within its double average crown diameter (Fig. 18). In the not-associated setting *Olea europaea subsp. cuspidata* is without any other shrubs (except other *Olea europaea subsp. cuspidata*) within the double average crown diameter (Fig. 19).

It was assumed that the root system has roughly the same expansion as the crown. Thus a shrub outside the double average crown size radius would not have a strong belowground effect on *Olea europaea subsp. cuspidata* in regard improved mycorrhization and nitrogen supply.

Fig. 18 Associated setting: *Olea europaea subsp. cuspidata* closely surrounded by other shrubs and trees (own work)

Fig. 19 Not-associated setting: *Olea europaea subsp. cuspidata* without other shrubs and trees within its double average crown diameter (own work)
Due to the difficult and densely vegetated terrain lying out a trajectory was not possible. To spread the samples over a larger area and thus include a wider range of micro-conditions we excluded every first and second *Olea europaea subsp. cuspidata* along the walked path while we took the third individual along the walked path for sampling (Fig. 20).

![Diagram](image)

*Fig. 20 Sample selection process (own work)*

To avoid taking a lot of samples from a single spot with a high number of individuals the setting grouped and not-grouped were defined. The not-grouped setting would have a single *Olea europaea subsp. cuspidata* without any other *Olea europaea subsp. cuspidata* within its double average crown size radius. The grouped setting would have and *Olea europaea subsp. cuspidata* surrounded by other *Olea europaea subsp. cuspidata* within its double average crown size radius. When we encountered a group, not the first *Olea europaea subsp. cuspidata* on the path, but the one farthest from the other *Olea europaea subsp. cuspidata* would be sampled, to minimize effects of competition for space on potential nurse shrubs.

When the third *Olea europaea subsp. cuspidata* along the path was determined and marked we measured height, widest crown part and narrowest crown part. Then we calculated the double average crown radius and recorded all shrubs within it in sketches, indicating the species and the dominance relative to other surrounding shrubs (Fig. 19). Then we took leaf samples of *Olea europaea subsp. cuspidata* and collected samples from all species recorded on the sketch. We measured the SPAD value for ten random *Olea europaea subsp. cuspidata*
leafs and ten random leafs for each collective sample of the surrounding shrubs. Then the leaves were stored in paper bags for further processing. Further we took a fine root sample (<2mm in diameter) of the *Olea europaea subsp. cuspidata* from the upper 20cm of soil. To avoid confusion with roots of other species we took only roots right next to the trunk, that were still attached to it. The roots were stored it in 50% ethanol.

Additionally we took leaf samples for $\delta^{15}$N, total leaf nitrogen and SPAD reference values from two adult trees. One was at the edge of the exclosure, the other within the nearby field. In total 39 leaf and 37 fine root samples from *Olea europaea subsp. cuspidata* were collected and analyzed. Of those, 27 samples were collected from *Olea europaea subsp. cuspidata* within the range of other shrubs (associated group) and 10 samples from *Olea europaea subsp. cuspidata* far from other shrubs (non-associated group).

Locals identified the recorded surrounding shrubs with common names, which we then used as clues to find the matching scientific names for the collected plant material.
7.6. Sample preparation

To enable a safe transport and for further analysis the leaf samples were air dried in the shade. We then ground the *Olea europaea subsp. cuspidata* leaf samples (pulverisette steel cups with seven balls for three minutes at 350 rpm) into a fine powder.

To conduct a $\delta^{15}N$ isotope analysis the machines must be calibrated for the expected range of % N. In order to do this we randomly picked seven *Olea europaea subsp. cuspidata* leaf samples (Nr. 3, 8, 19, 26, 28, 30, 32) and analyzed their % nitrogen content. It ranged between 0, 58% and 1, 55% nitrogen. With this range the sample weight calculator provided by Stable Isotope Facility estimated the optimal sample weight at 4,08mg.

4, 08 mg (+/- 0,002mg ) leaf powder of each *Olea europaea subsp. cuspidata* sample and four replicates were weighed into tin capsules, and analyzed at UC Davis Stable Isotope Facility, California. The *Olea europaea subsp. cuspidata* leaf samples were analyzed with an elemental analyzer (PDZ Europa ANCA-GSL) interfaced to a continuous flow isotope ration mass spectrometer (IRMS, PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). The long term standard deviation for this procedure is 0, 2 permil for $^{15}N$ (UC Davis Stable Isotope Facility 2016).

To enable safe transport and further analysis the root samples where stained with trepan blue and put onto slides for examination under a compound microscope.

The staining and preparing of the slides was conducted at the Department of Microbial, Cellular and Molecular Biology, College of Natural Sciences, Addis Ababa University, as follows (Fig. 21) (Brundrett 1996):

1. Wash with tap water
2. Autoclave for 15 minutes with 10% KOH at 120°C
3. Wash with tap water
4. Bleach for 15 minutes with a mixture of 2ml 0,25% NH$_4$OH and 30ml 10% H$_2$O$_2$
5. Wash with tap water
6. Acidify 10 minutes with 10% HCl
7. Drain HCl and stain with 0,05% trepan blue for 1h
8. Autoclave for 7 minutes at 120°C
9. Wash with tap water
11. Spread Polyvinyl-Lacto-Glycerol on slides and align root parts horizontally; after adding the coverslip crush the roots by gently pressing with another cover slide.
The root samples differed in length and due to the cooking process we lost some fine root material. Thus the root length per sample varied. To account for this variation we randomly applied 50 microscope grids per sample, which amount to 7,021cm of examined root length. The mycorrhiza colonization was assessed via a qualitative count of mycorrhizal organs that intersected the grid. The mycorrhizal organs were classified into arbuscules, vesicles, hyphae and unidentifiable elements (neither arbuscules, nor vesicles nor hyphae).

To account for improved identification skills after the first samples the first 10 root samples were resampled.

### 7.7. Statistical analysis

The potential nurse shrubs were analyzed with a cumulative count, indicating species ratio and ratio of dominant individuals of the three most common species.
The data related to *Olea europaea subsp. cuspidata* was tested for normal distribution and equal variance with the Shapiro-Wilk test and the Brown Forsythe test. Height, average crown size and number of arbuscules were not normally distributed and square root transformed. The number of hyphae was not normally distributed and sinus transformed. A One-way ANOVA was used to detect differences between the groups associated/not associated and dominant/not dominant. Vesicles were not normally transformed; a normal distribution through data transformation was not possible. A One-way ANOVA on ranks was used to detect differences between the groups associated/not associated and dominant/not dominant. A Correlation Analysis was performed and a Regression Analysis and a General Linear Model were used to determine the factors which influence height, average double crown size diameter, total leaf nitrogen and $^{15}$N content. One outlier (ID 32) was removed because it was sampled within a field and therefore probably strongly influenced by fertilizer. The root samples ID 4 and ID 18 were excluded from the mycorrhizal analysis because they were too short to apply 50 grids on different spots of the sample.
8. Results

Nurse shrubs

30 different shrub and tree species were recorded within the double average crown radius of all associated *Olea europaea subsp. cuspidata*. About 50% of the individuals were from six species: *Dodonea angustifolia, Carissa edulis, Clutia sp., Acacia sp., Calpurnia aurea* and *Vernonia sp.* (Fig. 22).

Of those nurse shrubs *Dodonea angustifolia* was present within the average crown radius in 25, *Carissa edulis* in 19 and *Clutia sp.* in 15 of 30 cases of associated *Olea europaea subsp. cuspidata*. Other species were present with fewer than half of the sampled, associated *Olea europaea subsp. cuspidata* (Fig. 23).

![Shrub and tree species determined within a circle of the double average crown radius of *Olea europaea subsp. cuspidata* growing in an exclosure site at Ambo Ber.](image1.png)

Fig. 22. Shrub and tree species determined within a circle of the double average crown radius of *Olea europaea subsp. cuspidata* growing in an exclosure site at Ambo Ber. Data shown are the percentage occurrence of each species as a total of all individuals determined. The identity of “Other” species is shown in Table 3.
Table 3. Other species within the double average crown radius of *Olea europaea subsp. cuspidata* growing in an exclosure site at Ambo Ber. Data shown are the percentage of occurrence of other species as a total of all determined individuals.

<table>
<thead>
<tr>
<th>Species</th>
<th>Percentage of occurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unidentified Species 2</td>
<td>4,07 %</td>
</tr>
<tr>
<td><em>Acacia saligna</em></td>
<td>2,91 %</td>
</tr>
<tr>
<td>Unknown Species 25</td>
<td>2,33 %</td>
</tr>
<tr>
<td><em>Hibiscus sp.</em></td>
<td>2,33 %</td>
</tr>
<tr>
<td><em>Croton macrostachys</em></td>
<td>1,74 %</td>
</tr>
<tr>
<td><em>Entarda abessynica</em></td>
<td>1,74 %</td>
</tr>
<tr>
<td><em>Jasminum abessynicum</em></td>
<td>2,33 %</td>
</tr>
<tr>
<td><em>Rhus glutinosa</em></td>
<td>2,33 %</td>
</tr>
<tr>
<td><em>Rosa abessynica</em></td>
<td>1,16 %</td>
</tr>
<tr>
<td><em>Acacia decurrens</em></td>
<td>1,16 %</td>
</tr>
<tr>
<td><em>Camaecytes palmensis</em></td>
<td>1,16 %</td>
</tr>
<tr>
<td><em>Hypericum quartinianum</em></td>
<td>1,16 %</td>
</tr>
<tr>
<td><em>Maytenus undata</em></td>
<td>1,16 %</td>
</tr>
<tr>
<td><em>Rumex nervosus</em></td>
<td>1,16 %</td>
</tr>
<tr>
<td><em>Myrsine africana</em></td>
<td>1,16 %</td>
</tr>
<tr>
<td><em>Cassia singueana</em></td>
<td>0,58 %</td>
</tr>
<tr>
<td><em>Ficus Tonningii</em></td>
<td>0,58 %</td>
</tr>
<tr>
<td><em>Schinus molle</em></td>
<td>0,58 %</td>
</tr>
<tr>
<td>Unidentified Species 10</td>
<td>0,58 %</td>
</tr>
<tr>
<td>Unidentified Species 14</td>
<td>0,58 %</td>
</tr>
<tr>
<td>Unidentified Species 16</td>
<td>0,58 %</td>
</tr>
</tbody>
</table>
Fig. 23. Nurse shrub species within the double average crown radius of associated *Olea europaea subsp. cuspidata* growing at an exclosure site at Ambo Ber. Bars show the number of cases in which the respective shrub or tree species was present.
Mycorrhization

Our samples showed vesicular arbuscular mycorrhiza only (Fig. 27, Fig. 28), no ectomycorrhizal structures were found. Associated *Olea europaea subsp. cuspidata* have the same number of vesicles, hyphae and arbuscules as not-associated *Olea europaea subsp. cuspidata* (Fig. 26 left side). Dominant *Olea europaea subsp. cuspidata* have the same number of vesicles, hyphae and arbuscules as not-dominant *Olea europaea subsp. cuspidata* (Fig. 26 right side).

![Diagram showing mean number of vesicles, hyphae and arbuscules of associated and not-associated Olea europaea subsp. cuspidata growing at the exclosure site at Ambo Ber. Bars show mean ± SE. Bars within a parameter not followed by the same indices within vesicles, hyphae or arbuscules are significantly different (p=0.05).](image)

Fig. 24. Mean number of vesicles, hyphae and arbuscules of associated and not-associated *Olea europaea subsp. cuspidata* growing at the exclosure site at Ambo Ber. Bars show mean ± SE. Bars within a parameter not followed by the same indices within vesicles, hyphae or arbuscules are significantly different (p=0.05).

![Images showing 0.14cm of root of Olea europaea subsp. cuspidata growing at Ambo Ber colonized by hyphae and vesicles (own work) and 0.07cm of root of Olea europaea subsp. cuspidata growing at Ambo Ber colonized by an arbuscule (own work).](images)

Fig. 26. 0.14cm of root of *Olea europaea subsp. cuspidata* growing at Ambo Ber colonized by hyphae and vesicles (own work)  
Fig. 25. 0.07cm of root of *Olea europaea subsp. cuspidata* growing at Ambo Ber colonized by an arbuscule (own work)
Plant size
Associated *Olea europaea subsp. cuspidata* are significantly higher and have a significantly wider average crown diameter than not-associated *Olea europaea subsp. cuspidata* (Fig. 24). Height increases significantly with the number of vesicles ($\alpha < 0.1$), but not with the number of arbuscules and hyphae (Fig. 25 left side). The average crown diameter increases significantly with the number of vesicles, but not with the number of hyphae and arbuscules (Fig. 25 right side).

![Graph showing plant height and crown diameter](image)

**Fig. 27.** Mean height of associated and not-associated *Olea europaea subsp. cuspidata* growing at the exclosure site at Ambo Ber. Bars show mean ± SE. Bars within a parameter not followed by the same indices are significantly different ($p=0.05$).
Fig. 28. Relationship between vesicles, hyphae or arbuscules, with plant height or average crown diameter of *Olea europaea*, growing at the exclosure site at Ambo Ber.
Nitrogen

Associated *Olea europaea subsp. cuspidata* have a significantly lower SPAD value than not-associated *Olea europaea subsp. cuspidata* but the same total leaf nitrogen and δ¹⁵N content (Fig. 31 left side). Further associated *Olea europaea subsp. cuspidata* have a higher variance in SPAD and total nitrogen content, but not in δ¹⁵N (Fig. 31 right side).

Dominant *Olea europaea subsp. cuspidata* the same total leaf nitrogen content, δ¹⁵N content and SPAD value as not-dominant *Olea europaea subsp. cuspidata*.

δ¹⁵N content increases as total leaf nitrogen content increases (Fig. 29). Also the reference trees and the outlier, which were excluded from the analysis, follow this trend. The δ¹⁵N of *Dodonea angustifolia*, the most common nurse shrub, was -1. 4‰ with a total nitrogen content of 1. 84%.

Neither total leaf nitrogen content nor δ¹⁵N content change with the number of vesicles, hyphae and arbuscules (Fig. 30).

The SPAD, total leaf nitrogen and δ¹⁵N reference values of the adult trees are listed in Table 4.
Fig. 29. SPAD value, total nitrogen and $\delta^{15}$N of leaves of associated and not-associated Olea europaea subsp. cuspidata, growing at the exclosure site at Ambo Ber. Shown are mean ± SE (left) and the data as a box plot (right). Bars not followed by the same indices are significantly different (p=0.05). The boxplots show median and 25th/75th percentile. Dots show included outliers.
Fig. 30 The relationship between total leaf nitrogen and $\delta^{15}$N of leaves of *Olea europaea subsp. cuspidata*, growing at the exclosure site at Ambo Ber. Shown are data from associated and not-associated trees, as well as adult trees growing outside the exclosure and the removed outlier.
Fig. 31: Relationship between vesicles, hyphae or arbuscules with δ¹⁵N or total leaf nitrogen content of Olea europaea subsp. cuspidata growing at the exclosure site at Ambo Ber.
<table>
<thead>
<tr>
<th></th>
<th>SPAD</th>
<th>Total leaf N (%)</th>
<th>$\delta^{15}$N (‰)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>adult tree 1</strong></td>
<td>70.3</td>
<td>1.953026</td>
<td>- 0.23</td>
</tr>
<tr>
<td><strong>adult tree 2</strong></td>
<td>74.4</td>
<td>1.939092</td>
<td>0.97</td>
</tr>
</tbody>
</table>

Table 4 SPAD, total leaf nitrogen and $\delta^{15}$N reference values of adult trees
Height decreases with increasing $\delta^{15}$N ($\alpha < 0.001$), total nitrogen content ($\alpha < 0.05$) and SPAD value ($\alpha < 0.05$) (Fig. 32 left side). Further the average crown diameter decreases with increasing $\delta^{15}$N ($\alpha < 0.1$). The average crown diameter does not change with increasing SPAD value and total nitrogen content (Fig. 32 right side).

Fig. 32. Relationship between $\delta^{15}$N, total leaf nitrogen or SPAD value with plant height or average crown diameter of *Olea europaea subsp. cuspidata*, growing at the exclosure site at Ambober.
The General Linear Model shows that, of all considered factors, $\delta^{15}$N is the most strongly related height. The other factors vesicles, hyphae, arbuscules and total nitrogen content cannot explain the differences between the groups (associated/not-associated and dominant/not-dominant) concerning height and average crown diameter (Fig. 33).

![General Linear Model of factors determining the plant height of Olea europaea subsp. cuspidata growing at the exclosure site at Ambober](image_url)

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected Model</td>
<td>138229.461</td>
<td>5</td>
<td>27645.892</td>
<td>4.773</td>
<td>.003</td>
</tr>
<tr>
<td>Intercept</td>
<td>2434.621</td>
<td>1</td>
<td>2434.621</td>
<td>.420</td>
<td>.522</td>
</tr>
<tr>
<td>$\delta^{15}$N</td>
<td>17078.280</td>
<td>1</td>
<td>17078.280</td>
<td>2.948</td>
<td>.096</td>
</tr>
<tr>
<td>res_vesicles</td>
<td>10264.823</td>
<td>1</td>
<td>10264.823</td>
<td>1.772</td>
<td>.193</td>
</tr>
<tr>
<td>res_arbuscules</td>
<td>424,430</td>
<td>1</td>
<td>424,430</td>
<td>.073</td>
<td>.788</td>
</tr>
<tr>
<td>N_per_sample</td>
<td>2519.761</td>
<td>1</td>
<td>2519.761</td>
<td>.435</td>
<td>.515</td>
</tr>
<tr>
<td>association</td>
<td>23604.970</td>
<td>1</td>
<td>23604.970</td>
<td>4.075</td>
<td>.053</td>
</tr>
<tr>
<td>Error</td>
<td>173769.512</td>
<td>30</td>
<td>5792.317</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>987957.000</td>
<td>36</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>311998.972</td>
<td>35</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. R Squared = .443 (Adjusted R Squared = .350)

Fig. 33 General Linear Model of factors determining the plant height of Olea europaea subsp. cuspidata growing at the exclosure site at Ambober
9. Discussion

The most common nurse shrubs at the exclosure in Ambo Ber - *Dodonea angustifolia*, *Carissa edulis* and *Clutia sp.* - belong to different plant families (Table 4), the Sapindaceae, the Apocynaceae and the Euphorbiaceae. *Dodonea angustifolia* is a shrub which grows up to 3m tall and occurs on grassland, secondary forest, recently cleared forest and overgrazed land (Hedberg, Edwards 1989b). *Carissa edulis* usually grows in Acacia woodland and riverine fringing vegetation. Even though it has long spines it is grazed vigorously (Hedberg 2003). *Clutia sp.* is a shrub which grows as a small tree or shrub. It occurs in evergreen bushland or open, deciduous Juniperus forests and on disturbed sites (Edwards et al. 1995a).

Therefore the preferred habitats and traits of *Dodonea angustifolia* and *Clutia sp.* seem to reflect the site history of forest clearance and overgrazing.

None of these shrubs or their families are known for fixing nitrogen. The literature does not mention any nitrogen fixing abilities of *Dodonea angustifolia*, *Carissa edulis* or *Clutia sp.* (Hedberg 2003; Edwards et al. 1995b; Hedberg, Edwards 1989a), but there is too little information to rule out the possibility that they fix. Therefore more research on potential nitrogen fixing abilities of *Dodonea angustifolia*, *Carissa edulis* and *Clutia sp.* is necessary.

Little else is known about *Dodonea angustifolia*, *Carissa edulis* and *Clutia sp.*. Therefore, to determine the potential facilitative effects of these species, other than nitrogen fixation, further research is needed to confirm specific shrub traits.

Other common shrubs inside the exclosure such as *Acacia sp.* and *Calpurnia aurea* belong to the Fabaceae and are potentially nitrogen fixing. Other potential nitrogen fixers within the exclosure are *Senna didymobotria*, *Acacia saligna*, *Entarda abessynica*, *Acacia decurrens*, *Camaecytes palmensis* and *Cassia sanguinea*. However these species occur in less than 50% of the cases.

Table 5 Plant family and nitrogen fixation of potential nurse shrubs (Hedberg 2003; Edwards et al. 1995a; Hedberg, Edwards 1989b)

<table>
<thead>
<tr>
<th>Species</th>
<th>Family</th>
<th>N-fixing according to literature</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Dodonea angustifolia</em></td>
<td>Sapindaceae</td>
<td>No</td>
</tr>
<tr>
<td><em>Carissa edulis</em></td>
<td>Apocynaceae</td>
<td>No</td>
</tr>
<tr>
<td><em>Clutia sp.</em></td>
<td>Euphorbiaceae</td>
<td>No</td>
</tr>
<tr>
<td><em>Acacia sp.</em></td>
<td>Fabaceae</td>
<td>Potentially fixing</td>
</tr>
<tr>
<td>Species</td>
<td>Family</td>
<td>Status</td>
</tr>
<tr>
<td>-------------------------</td>
<td>----------</td>
<td>------------------</td>
</tr>
<tr>
<td>Calpurnia aurea</td>
<td>Fabaceae</td>
<td>Potentially fixing</td>
</tr>
<tr>
<td>Senna didymobotria</td>
<td>Fabaceae</td>
<td>Potentially fixing</td>
</tr>
<tr>
<td>Acacia saligna</td>
<td>Fabaceae</td>
<td>Potentially fixing</td>
</tr>
<tr>
<td>Entarda abessynica</td>
<td>Fabaceae</td>
<td>Potentially fixing</td>
</tr>
<tr>
<td>Acacia decurrens</td>
<td>Fabaceae</td>
<td>Potentially fixing</td>
</tr>
<tr>
<td>Camaecytes palmensis</td>
<td>Fabaceae</td>
<td>Potentially fixing</td>
</tr>
<tr>
<td>Cassia sanguinea</td>
<td>Fabaceae</td>
<td>Potentially fixing</td>
</tr>
</tbody>
</table>

The difference in height and average crown diameter between the groups suggests that *Olea europaea subsp. cuspidata* growing beneath nurse shrubs benefits from improved conditions. These improved conditions could be related to, as our data indicated, improved mycorrhization as plant height increased with the number of vesicles per cm of root. Research on *Olea europaea subsp. cuspidata* has shown that plants inoculated with *Glomus intraradices* had a 50% higher photosynthetic rate, higher water use efficiency, increased plant height and stem diameter (Querejeta et al. 2003; Seifi et al. 2014). However we could not show that *Olea europaea subsp. cuspidata* associated with nurse shrubs has higher mycorrhization in comparison to none associated individuals. Nevertheless this could be the case. The similar mycorrhization of groups in our case might be due to the sampling period. Arbuscules have an estimated life span of 4-5 days (Bonfante, Genre 2010) and persist only for a few weeks, which is why the samples should be taken during periods favoring mycorrhizal activity (Brundrett, Kendrick 1990; Brundrett 1996). Our samples however, were taken at the end of the dry season. Arbuscles could have already been decayed while the formation of new arbuscles was restricted by dry soil conditions. Therefore any differences between *Olea europaea subsp. cuspidata* associated with nurse shrubs and without nurse shrubs might have been erased.

*Olea europaea subsp. cuspidata* has no ectomycorrhizal structures, arbuscular mycorrhiza is dominant (Wubet et al. 2003). Wubet et al (2003) found arbuscular colonization between 51 – 75%, vesicular colonization between 6 – 25% and hyphal colonization between 76 – 100%. Further the dominance of oval or elongated vesicles suggested the dominance of the species of the genus *Glomus*. 
Our results confirmed arbuscular mycorrhiza as a symbiont, but the dominant organs were vesicles, not arbuscules. As mentioned above the low number of arbuscles in comparison to the number of vesicles might be due to the sampling period. However a low frequency of arbuscules also indicates ineffective nutrient exchange, while high vesicular colonization is a potential indicator of fungal resource hoarding. High allocation to vesicles is prominent under high external nutrient conditions, when hosts are less dependent on fungal partners for nutrient uptake (Denison, Kiers 2011). Furthermore high amounts of inorganic phosphorous can lead to active inhibition of arbuscule development by plants (Breuillin et al. 2010). Therefore the high number of vesicles suggests nutrient abundance at the exclosure in Ambo Ber.

However, high amounts of phosphatase at the exclosure in Ambo Ber suggest nutrient deficiency, at least in regard to phosphorous (unpublished data). In order to confirm the cause for the low number of arbuscules, whether it reflects nutrient abundance or the sampling period, further research is needed.

The similar nitrogen content between groups suggests that there are no differences in nitrogen supply between groups and therefore that there is no effect of nurse shrubs on nitrogen supply. This indicates that if nitrogen fixing nurse shrubs are involved, their facilitation effect does not influence the nitrogen supply of nearby Olea europaea subsp. cuspidata. It further suggests that the difference in size between associated and not-associated Olea europaea subsp. cuspidata is caused by a factor other than nitrogen availability.

However the SPAD readings of not-associated Olea europaea subsp. cuspidata are significantly higher than those of associated individuals. Apart from measuring nitrogen content SPAD measurements can be used to determine chlorophyll a content and predict the net assimilation rate (Boussadia et al. 2011). This suggests that both groups had the same nitrogen supply but not-associated Olea europaea subsp. cuspidata used their nitrogen in a different, maybe more efficient way.

The strong correlation of total leaf nitrogen and SPAD measurements with height implies that this effect is also related to plant age or size.

Shading tends to lower the chlorophyll a content of Olea europaea (Nanos, Ilias 2007; Casas et al. 2011). However the high SPAD value, and therefore the high chlorophyll a content, of small associated Olea europaea subsp. cuspidata suggests, that shading by potential nurse shrubs is a negligible effect in the exclosure in Ambo Ber.
Nurse plants can improve growth (Molina-Montenegro et al. 2016; Torroba-Balmori et al. 2015). However, the positive effect on growth can decline within the first years after germination (Torroba-Balmori et al. 2015) and most studies relate the positive effect on growth to improved microclimate and physical protection (Torroba-Balmori et al. 2015; Molina-Montenegro et al. 2016; Liu et al. 2014). Research has shown that nurse plants can increase soil nutrients in comparison to open patches (Noumi et al. 2015; Liu et al. 2014; Liu, Guo 2012; Abiyu 2012) and that a significant change of δ₁⁵N of a non-N₂-fixing species next to a N₂-fixing species could indicate a transfer of fixed nitrogen from one species to another (van Kessel et al. 1994; Høgh-Jensen, Schjoerring 2000; Saitoh et al. 2006). However, with regard to direct nitrogen transfer little research has been done on the specific mechanisms of directed nitrogen transfer from nurse plant to nursed plant.

Nurse shrubs can provide microsites for mycorrhizal propagules and ¹⁵N transfer is significantly increased when plants are mycorrhizal (Miller 1987; Williams 2012; He et al. 2003). But our results from Ambo Ber on mycorrhization suggest that nurse shrubs neither function as providers for microsites, nor as nitrogen suppliers through directed mycorrhizal transfer.

However results on directed nitrogen transfer between field grown plants have been inconclusive (He et al. 2003; Hamel et al. 1991). Therefore a pot experiment with *Olea europaea subsp. cuspidata* and potential nurse shrubs is needed to confirm this.

Nitrogen fixers tend to have a higher amount of total leaf nitrogen than non-fixing species (Högberg, Alexander 1995; Hoogmoed et al. 2014). Therefore, even if a direct transfer of nitrogen is unlikely, an indirect transfer of nitrogen through the decay of enriched leaves or leaching from enriched leaves could still be possible.

Abiyu (2012) compared foliar nutrient content of potential nurse shrub species in the exclosure in Ambo Ber and Taragadam, present in 2007 (Table 6) and found *Dodonea angustifolia* to be in the lower range of foliar nitrogen contents. He found the soils under nurse shrubs enriched in nitrogen in comparison to plots between shrubs. However soil under *Dodonea angustifolia* had the lowest soil nitrogen content among the analyzed plots. Further our analysis revealed that the total leaf nitrogen content of *Dodonea angustifolia* contains only 0.3% more nitrogen than the average *Olea europaea subsp. cuspidata*. Therefore, the indirect effect of *Dodonea angustifolia* on the nitrogen supply of *Olea europaea subsp. cuspidata* is probably negligible.
Table 6 Soil under potential nurse shrubs and foliar nitrogen content of potential nurse shrubs in the exclosure at Ambo Ber (Abiyu 2012)

<table>
<thead>
<tr>
<th>Species</th>
<th>Foliar N (mg/g)</th>
<th>Soil N under nurse shrubs (mg/g)</th>
<th>Soil N between nurse shrubs (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Croton</td>
<td>30.8</td>
<td>3.83</td>
<td>2.35</td>
</tr>
<tr>
<td>Rhus</td>
<td>26.2</td>
<td>5.62</td>
<td>3.57</td>
</tr>
<tr>
<td>Vernonia</td>
<td>25.7</td>
<td>3.84</td>
<td>1.18</td>
</tr>
<tr>
<td>Otostegia</td>
<td>23.1</td>
<td>2.91</td>
<td>1.77</td>
</tr>
<tr>
<td>Dodonea</td>
<td>22.9</td>
<td>1.69</td>
<td>1.70</td>
</tr>
<tr>
<td>Maytenus</td>
<td>17.4</td>
<td>5.54</td>
<td>4.31</td>
</tr>
</tbody>
</table>

The δ¹⁵N signatures from shoots of N-fixing plants can range from as high as +1.9‰ to as low as -4.6‰, but most values range between -1‰ and -2‰ (Boddey et al. 2000). This indicates that Dodonea angustifolia, with a δ¹⁵N signature of -1.4‰, could be fixing nitrogen. However, more research is needed to confirm this.

Further the high ¹⁵N content of Dodonea angustifolia poses the question why associated Olea europaea subsp. cuspidata have a much lower ¹⁵N content. Should not the ¹⁵N signal of Dodonea angustifolia raise the ¹⁵N signal of associated Olea europaea subsp. cuspidata? This further supports the suggestion that an increased transfer of fixed, ¹⁵N-enriched nitrogen, from Dodonea angustifolia to Olea europaea subsp. cuspidata, both directly and indirectly, is unlikely. But to confirm this more research is needed. First of all a pot experiment should be conducted to examine the isolated effect the Dodonea angustifolia ¹⁵N signal on the Olea europaea subsp. cuspidata ¹⁵N signal and second, field samples of potential ¹⁵N sources should be gathered and analysed.

This leaves the question as to why associated Olea europaea subsp. cuspidata have a slightly lower ¹⁵N signal than not-associated Olea europaea subsp. cuspidata.

The slightly lower δ¹⁵N content of the not-associated group could be explained by fractionated nitrogen inputs by passing ungulates in the area were most of the not-associated Olea europaea subsp. cuspidata were sampled. Nitrogen leaving an animal via urine is depleted in ¹⁵N and could lower the δ¹⁵N of nearby Olea europaea subsp. cuspidata. However recently deposed urine can also be a site of NH₃ volatilization and subsequent enrichment (Högberg 1997).

Another explanation for the slightly different δ¹⁵N contents could be the influence of fertilizer from the nearby field. Ethiopian farmers are recommended to use urea and DAP (100/kg/ha
Due to price and distribution problems the input is probably much lower (Spielman et al. 2010) and the $\delta^{15}N$ signature of fertilizers depends on the manufacturer (Bateman, Kelly 2007). The $\delta^{15}N$ signature of urea supplied by Yara International, a company that has supplied considerable amounts of fertilizer to the Ethiopian government in the past years, is – 5.9‰ (Hagen 2016; Fasika 2015; Bateman, Kelly 2007; Ariz et al. 2015). Since the fertilizer is usually applied during the rain time the lower signal of associated *Olea europaea subsp. cuspidata* could be caused by relocated urea or DAP with a negative $^{15}N$ signal. However, the outlier ($\delta^{15}N - 0.76‰$) and the mother trees ($\delta^{15}N -0.22‰$ and $0.96‰$), growing inside and at the edge of the field, show comparably high $\delta^{15}N$ contents.

Another factor for interpreting $\delta^{15}N$ contents are metabolic processes such as translocation or volatilization, which might cause variations within different plant parts. Therefore the $\delta^{15}N$ natural abundance method should only be used, when the $\delta^{15}N$ of foliage of reference groups deviates more than 5‰ from that of nitrogen derived by N2-fixation, if there are no complementary data on nitrogen pool sizes, patterns of nitrogen transformations or root distribution (Högberg 1997). The difference between the associated and the not-associated group is less than 5‰ and without complementary data. Therefore our results on $\delta^{15}N$ cannot be explained without reserve.

Due to the similarities in total leaf nitrogen, $\delta^{15}N$ content and mycorrhization of associated and not-associated *Olea europaea subsp. cuspidata* the growth at the exclosure in Ambo Ber is probably influenced by a factor other than nitrogen.

This factor could be browsing. Browsing can influence the total leaf nitrogen content. A study on *Olea europaea var. sylvestris* and *Olea europaea var. europaea* found that leaves of protected trees had higher leaf nitrogen contents than unprotected trees, which had lower total leaf nitrogen contents (Massei, Hartley 2000).

Nurse shrubs can provide some protection from browsing (Aerts et al. 2007). However, the total leaf nitrogen and $^{15}N$ content of *Olea europaea subsp. cuspidata* at Ambo Ber changes rather with tree size than with association to nurse shrubs. Therefore protected trees do not necessarily have higher total leaf nitrogen contents.

If a tree is limited by browsing, instead of nitrogen, it can continue to take up nitrogen, while distributing the nitrogen among fewer and smaller leaves. In contrast, unbrowsed trees, with access the same nitrogen sources have to distribute them among more and larger leaves.
Browsing has strong negative effects on seedling growth (Alemayehu 2007). Most non-associated and small *Olea europaea subsp. cuspidata* at Ambo Ber showed small, almost round leaves, shortened shoots and a dense habitus which indicates browsing (Fig. 34, Fig. 35) (Massei et al. 2000; Aerts et al. 2008).

At low browsing pressures seedlings below nurse shrubs are less likely to be eaten or damaged than in bare patches, at high browsing pressures this effect disappears. Accordingly nurse shrubs alone cannot protect seedlings efficiently against browsing (Aerts et al. 2007). Therefore browsing, mitigated by nurse shrubs, could explain the successful growth of some *Olea europaea subsp. cuspidata*, while others stay small, regardless of sufficient nitrogen supply and mycorrhization (Fig. 36). This suggests that the influence of nurse shrubs on the growth and establishment of *Olea europaea subsp. cuspidata* in the exclosure at Ambo Ber is not species specific, but general.
To gain deeper understanding and clarify existing results about the relation between potential nurse shrubs and *Olea europaea subsp. cuspidata* at the exclosure in Ambo Ber further research is needed. Sampling with a smaller sampling radius, a determination of the mycorrhiza species, a δ^{15}N analysis of all potential nurse shrubs, a pot experiment to determine the B-value and additional data on soil δ^{15}N content would be needed to expand the present interpretation of results.
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Appendix

17 of 18 Agroclimatic zones (Bekele-Tesemma, Tengnäs 2007)

1. DRY BEREHA = Dry Hot-lowlands
2. MOIST BEREHA = Moist Hot-lowlands
3. DRY KOLLA = Dry Lowlands
4. MOIST KOLLA= Moist Lowlands
5. WET KOLLA = Wet Lowlands
6. DRY WEYNA DEGA = Dry Mid-highlands
7. MOIST WEYNA DEGA= Moist Mid-highlands
8. WET WEYNA DEGA= Wet Mid-highlands
9. DRY DEGA = Dry Highlands
10. MOIST DEGA = Moist Highlands
11. WET DEGA = Wet Highlands
12. DRY WURCH = Dry Frost zones
13. MOIST WURCH = Moist Frost zones
14. WET WURCH =Wet Frost Zones
15. DRY ALPINE WURCH = Dry Alpina-frost Zones
16. MOIST ALPINE WURCH = Moist Alpina-frost zones
17. WET ALPINE WURCH = Wet Alpina-frost zone