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A Theoretical Development and Field Test of a Horizontal Line Sampling (HLS) in Coppice Forests

Theoretische Entwicklung und Feldtest einer horizontalen Linienstichprobenmethode (HLS) für Ausschlagwälder

Habib Ramezani^{1*}, Nastaran Nazariani²

- Keywords:
 cost-efficient design, forest inventory, open sparse oak forest,

 Quercus brantii, Zagros forest
- Schlüsselbegriffe: kosteneffizientes Design, Waldinventur, offener lichter Eichenwald, *Quercus brantii*, Zagros Wald

Abstract

If available forest inventory techniques or measurement instruments are not satisfying or appropriate for monitoring a particulare forest, options include developing a new inventory technique and/or modifying an available instrument. In this study, we present a new application of horizontal line sampling (HLS) in combination with a crown relascope as a potential alternative to line intersect sampling (LIS) to monitor coppice forests (CF). In CFs, the stem basal area relascope is not applicable, since trees often lack distinct stems and are not visible at breast height. Our study was conducted in an open oak-dominated CF in western Iran and we focussed on two forest attributes: above-ground biomass (*AGB*) and the total number of trees (*N*). We assessed the field performance of HLS and LIS in CFs and evaluate various line transect lengths and crown relascope angles. Our results indicate that longer line transects (80 m) produce smaller relative sampling errors compared to shorter ones (20 m) and smaller relascope angles (36.8°) are more precise than larger ones (67.4°). In terms of cost-efficiency, HLS is superior or equivalent to LIS in some cases. While HLS and LIS

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gave different estimates of AGB and N, the difference was not statistically significant at the 95% level. Both relascope angle and line transect length impact the efficiency of HLS. Our study provides evidence that this technique can be useful for monitoring forest conditions.

Zusammenfassung

Wenn verfügbare Waldinventurtechniken und/oder Messinstrumente nicht geeignet sind, um ein ausgewähltes Waldgebiet zu beobachten, dann ist eine Lösung, neue Inventurtechniken zu entwickeln und/oder verfügbare Messinstrumente zu adaptieren. In dieser Studie stellen wir eine neue Anwendung der horizontalen Linienstichprobe (HLS) in Kombination mit einem Kronenrelaskop vor. Als potenzielle Alternative zur Linienkreuzungsstichprobe (LIS) testen wir hier die HLS in Ausschlagwäldern (CF). In niederwaldähnlichen Ausschlagwäldern ist das Stammgrundflächenrelaskop nicht anwendbar, da Bäume oft keine ausgeprägten Stämme haben und in Brusthöhe oft nicht sichtbar sind. Die Studie wurde in einem offenen und von Eichen dominierten Ausschlagwald im westlichen Iran durchgeführt. Die Studie fokussiert sich auf zwei Waldattribute, oberrirdische Biomasse (AGB) und die Gesamtzahl der Bäume (N). Wir bewerten die Leistungsfähigkeit von HLS- und LIS-Inventurtechniken in CF sowie den Effekt verschiedener Transektlängen und Kronenrelaskopwinkel. Unsere Ergebnisse zeigen, dass eine längere Transektlänge (80 m) einen kleineren relativen Stichprobenfehler aufweist als die kürzere Transektlänge (20 m) und ein kleinerer Relaskopwinkel (36,8°) präziser ist als ein größerer Relaskopwinkel (67,4°). Hinsichtlich der Kosteneffizienz ist HLS der LIS überlegen oder in einigen Fällen gleichwertig. HLS und LIS ergaben unterschiedliche Schätzungen von AGB und N, aber der Unterschied war statistisch nicht signifikant auf dem 95-%-Niveau. Sowohl Relaskopwinkel als auch Transektlänge haben Auswirkungen auf die Effizienz des HLS. Unsere Studie liefert einen Beleg, dass die HLS-Technik für das Monitoring des Waldzustandes hilfreich sein kann.

1 Introduction

A larger number of forest inventory techniques have been proposed to collect the necessary information for forest management planning. In forest inventories, the primary objective is to estimate the parameters with the highest possible accuracy/precision and at a reasonable cost (Kershaw *et al.* 2016). However, a given forest inventory technique and/or measurement instrument may not be efficient when applying it in different situations and for various purposes. The characteristics of the forest, survey's objective and costs determine the appropriate inventory technique and measurement to be used. For instance, Schreuder *et al.* (1987) suggested

that HPS is a highly effective technique in estimating stand-level basal area, but it may be less effective in estimating other parameters such as tree density. To improve precision in the estimation of snags, Ducey *et al.* (2002) utilized a combination of horizontal point sampling (HPS) and horizontal line sampling (HLS). Hence, developing a new inventory technique or modifying the currently used measurement instrument to suit various situations is desirable.

Unlike forests, where a stand is characterized by single-stemmed trees that originate from seed, there is another form of forest around the world, so-called coppice forest (CF) (Scolastri et al. 2017). CF consist of trees that have mainly been regenerated from shoots and a small percentage of seed-originated trees (Scolastri *et al.* 2017; Iranmanesh *et al.* 2019). Young tree stems are repeatedly cut down to near ground level, resulting in a stool. These stands are formed through asexually/vegetatively sprouting, which is the most common means of regeneration (Vollmuth 2022).

CF are an important renewable resource, with the potential to provide products and services such as non-wood products, protection and heritage ecosystem services. While seed-originated trees have short stems and large crowns (Scolastri *et al.* 2017), in CFs shoots-regenerated trees often lack a distant stem. Vollmuth (2022) conducted a survey in Germany's coppice forest. The author concluded that coppice forests have high overall biodiversity and that they provide habitats for numerous light-demanding species, that are endangered today.

The stem basal area relascope is a commonly used instrument in forest inventory for estimating variables such as basal area (BA) and volume (Bitterlich 1984; Lynch *et al.* 2021). However, in CFs, where trees generated through sprouting and do not have distinct stems, the stem basal area relascope may not be as effective due to the invisibility of the stems in breast height. To address this issue, the crown relascope (CrR, Stenberg *et al.* 2008) may be an efficient alternative. The CrR is an extension of the stem basal area relascope. CrR estimates vertically projected crown area on the ground or crown cover using tree crown diameter as the criterion for selection. However, the use of CrR requires a larger basal area factor known as the crown basal area factor (CBAF).

Line intersect sampling (LIS) is a well-known and efficient technique for sampling different kinds of features (Kaiser 1983, DeVries 1986, Skidmore and Turner 1992). It has been used for measuring the area of two-dimensional objects (Battles *et al.* 1996, Ramezani & Holm 2011), in estimating total length of linear features (*e.g.*, Matérn 1964, Corona et al. 2004, Ramezani & Holm 2011), and also to estimate the total number of objects (Gregoire & Valentine 2008). However, LIS may not be efficient for sparse populations (Cooper 1957; Ståhl 1997). In such a population, we may encounter one of the two following situations: either there is a need for a large sample size, or the estimated sampling error (or variance) will be large (Ducey *et al.* 2002). Horizontal line sampling (HLS) was originally developed by Strand (1957) to estimate attributes of standing trees. Lynch (2006) and Marquardt *et al.* (2010) applied HLS to estimate total amounts of forest attributes in riparian forests. Rice *et al.* (2014) found that HLS was also effective in partially harvested stands, and it has also been used in forest inventory in Taiwan (Yang & Chao 1987). In HLS, Bitterlich sampling (1984) and LIS features are combined and a stem basal area relascope is often used in estimating forest parameters (Schreuder *et al.* 1987; Gregoire & Valentine 2008). For quantifying coarse woody debris in a sparse population, Ståhl (1997) developed a transect relascope (TR), which combines LIS and a wide-angle relascope instead of the stem basal area relascope. The method section (2.2) provides detailed description of the estimators and inclusion probabilities of both LIS and HLS.

In previous examples of HLS applications, the same instrument – a stem basal area relascope – was generally used as in horizontal point sampling (HPS). However, this study presents a new application of HLS in combination with CrR in a coppice forest. The study compares the field performance, precision, and time efficiency of HLS and LIS inventory techniques. The main objective is to estimate forest attributes such as above-ground biomass (*AGB*) and the total number of trees (*N*) in a CF where trees do not have distinct stems. Additionally, the study assesses the performance of HLS and LIS at varying transect lengths (80, 40, and 20 m) and relascope angles (36.8, 53.1, and 67.4°). While sample size has an obvious effect on the precision of estimates, it is not examined in this study.

2 Material and methods

2.1 Study area

The study was conducted in an open, oak-dominated coppice forest (CF) (*Quercus brantii*) in Lorestan province, western Iran (Zagros forests, see Fig. 1). *Quercus brantii* is a significant tree species in the western forests of Iran. These oak stands are often found mixed with *Juniperus excelsa M. Bieb, Amygdalus scoparia Spach., Pistacia atlantica Desf.* and *Acer monspessulanum* L. Due to human impact, only 7% of the oak forests are considered forests, while the remaining 93% are in coppice form (Iranmanesh *et al.* 2019; Mahdavi *et al.* 2020). We note that according to FAO, forest land is defined as land covering more than 0.5 hectares with trees higher than 5 meters and a canopy cover of more than 10 percent. Zagros forests cover an area of approximately 5 million hectares and are classified as semi-arid, accounting for 40% of Iran's forests. The elevation of the study area ranges from 1860 m to approximately 2070 m above sea level, with a mean annual precipitation of 530.15 mm and a mean annual temperature of 18.3° C (Sagheb-Talebi *et al.* 2003). Zagros forests are non-timber-oriented and provide valuable ecosystem services such as water resources, climate regulation, and biodiversity conservation. The social economy of Zagros forests is closely linked

to the sustainable use and management of the forest ecosystem, with activities including ecotourism, handicrafts, and value-added processing of forest products (Salehi 2009; Riyahi 2010). CFs have been a crucial source of energy in the form of firewood and charcoal for rural people for thousands of years. Animal husbandry is a common land use practice in the Zagros forest (Safari & Sohrabi 2019). Fire frequently occurs in the Zagros forest during the summer and early autumn months. This fire season coincides with the dry and hot periods. Wildfires play a natural role in maintaining forest health and biodiversity. Some tree species in the Zagros forest have adapted to fire and depend on it for their regeneration and growth (Javanmiri pour 2021).



Figure 1: Location of the study area; a) overview of Iran; b) province; c) distance to nearby city; and d) the study area in an open oak stand.

Abbildung 1: Lage des Untersuchungsgebiets; a) Überblick über Iran, b) Provinz, c) naheliegende Stadt und d) das Untersuchungsgebiet in einem offenen Eichenbestand.

2.2 Sampling design

In this study, we laid out a systematic array of 25 m × 25 m line grids over the study area (about 10.4 ha) in a GIS environment. A total of 166 points were located in the study area, of which 30 were randomly selected and established in the field using a global positioning system (GPS). These locations served as the midpoints of the line transects. The transects were laid out using measuring tape and a hand compass, with orientations determined according to a uniform distribution [0, 2π]. To avoid boundary bias, we applied the walk-back method (Gregoire & Valentine 2008, p. 299), where if a boundary is intersected by a transect, the intersection point with the boundary serves as the starting point and we walk back towards the original sampling location.

We made a simple crown relascope (CrR) using wooden pieces and string (Fig. 2). A common and practical way to determine the desired angle for constructing a relascope is to use a reach-to-width ratio (R:W) (Table 1). Using CrR, the crown basal area factor (CBAF, m² ha⁻¹) is the amount of crown basal area per hectare that each sampled tree represents. Under CrR, crown cover (CrC) can be estimated by counting the average number of tree crowns (t) that appear wider than the crown relascope's angle and multiplying the result by a predefined CBAF (m² ha⁻¹), CrC = t × CBAF. For instance, CBAF 2500 (m² ha⁻¹) means that one tallied tree corresponds to 2500 m² of crown cover area per hectare.



Figure 2: Illustration of a crown relascope with reach-to-width 1:1 ratio (relascope angle 53.1°) and crown basal area factor 2500 (m² ha⁻¹). W and R are relascope width and distance length of string, respectively.

Abbildung 2: Darstellung eines Kronenrelaskops mit einem Verhältnis von Reichweite zu Breite von 1:1 (Relaskopwinkel 53,1°) und einem Kronengrundflächenfaktor von 2500 (m² ha⁻¹). W und R sind die Schlitzlänge und die Länge des Fadens des Relaskops.

Table 1: Reach-to-width ratio (R:W) and crown basal area factor (CBAF) for three HLS designs. $tan(V/2)^2 = (W/2)^2/(R)^2 = k$, and CBAF (m²ha⁻¹)=10000×k.

Tabelle 1: Verhältnis von Reichweite zu Breite (R:W) und Kronengrundflächenfaktor (CBAF) für drei HLS-Designs. $tan(V/2)^2=(W/2)^2/(R)^2=k$, und CBAF (m² ha⁻¹)=10000×k.

Relascope angle (°)	R (cm)	W (cm)	R:W	CBAF (m ² ha ⁻¹)
36.8 (V ₁)	50	33.3	1.5:1	1106
53.1 (V ₂)	50	50	1:1	2500
67.4 (V ₃)	50	66.7	1:1.3	4448

The next section provides a brief overview of the inclusion probabilities of trees when using LIS and HLS, as well as the estimators associated with these inventory techniques.

a) Line intersect sampling (LIS)

In LIS, the surveyor walked along the line transects and an object (tree) was included in the sample if the tree crown projected on the ground crossing a line transect. Then, the crown diameter of the selected trees was measured using measuring tape.

In a random sampling design (as our case), the inclusion probability of tree *i* ($\pi_{i,LIS}$), is:

$$\pi_{i,LIS} = \frac{cd_i \times l}{10^4 A} \tag{1}$$

where cd_i (m) is the tree crown diameter of tree *i*, *l* is transect length (m), and *A* is the total area (ha) (see Fig. 3, *right*).

The *Horvitz-Thompson* (*HT*) estimator of LIS to estimate the total above-ground biomass (*AGB*) for a single sampling unit, *j* (line transect) is

$$\hat{\tau}_{AGB,LIS,j} = \frac{10^4 A}{l} \sum_{i=1}^{m} \frac{AGB_i}{cd_i} \qquad (2)$$

and to estimate the total number of trees (N) the estimator is

$$\hat{\tau}_{N,LIS,j} = \frac{10^4 A}{l} \sum_{i=1}^{m} \frac{1}{cd_i}$$
(3)

where, AGB_i (kg) is the above-ground biomass of tree *i*, and *m* is the total number of trees sampled in the *j* th line.

A general *HT* estimator for the population total in a sample of *n* lines (the averaging across *n* sampling units) is

$$\hat{\tau}_{LIS} = \frac{1}{n} \sum_{j=1}^{n} \hat{\tau}_{LIS,j} \tag{4}$$



Figure 3: An illustration of the inclusion probabilities, under random sampling design, in the LIS and HLS cases. cd_i is corresponding the tree crown diameter tree i, r_i is corresponding crown radius of tree i, R_i is limiting distance of tree i, l is transect length, v is the fixed sighting angle of the relascope and a_i is the inclusion area of tree i. The line intersects sampling (LIS, right), the horizontal line sampling (HLS, left).

Abbildung 3: Eine Illustration der Einbeziehungswahrscheinlichkeiten bei zufälligem Stichprobendesign in den Fällen LIS und HLS. *cd*_i entspricht dem Kronendurchmesser des Baumes i, r_i entspricht dem Kronenradius des Baumes i, R_i ist der Grenzabstand des Baumes i, list die Transektlänge, v ist der feste Sichtwinkel des Relaskops und a_i ist die Einbeziehungsfläche des Baumes i. Die Linie schneidet die Stichprobe (LIS, rechts), die horizontale Linie Stichprobe (HLS, links).

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b) Horizontal line sampling (HLS)

In HLS, crown relascope (Fig. 2) was used when the same surveyor walking along the same line transects as in LIS. The surveyor sighted both sides of the transects and viewed all candidate trees through the relascope angle. Any tree with crown diameter greater than the width of the projected angle from the relascope was chosen as a sample tree. In this way, trees were sampled with probability proportional to their crown basal area. Note that we adopted modified sausage sampling (Gregoire & Valentine 2008, p 362), where trees selected from the same locations were omitted. Thus, in such a procedure, the inclusion probability is the same as under the conventional HLS.

Under HLS procedure, tree *i*, is selected if its perpendicular distance from the transect is within a limiting distance (R_i). In contrast to LIS, a tree is selected into a horizontal line sample without crossing directly by line transects. Under HLS, the inclusion probability of tree i ($\pi_{i,HLS}$), in a random sampling design, is (see Fig. 3, *left*):

$$\pi_{i,HLS} = 2R_i \times l \tag{5}$$

where R_i (m) is the limiting distance from which a tree appears exactly as wide as the measurement instrument that defines the sighting angle. According to Stenberg *et al.* (2008), R_i for crown relascope can be calculated as:

$$R_i = \frac{r_i}{\tan(V/2)} \tag{6}$$

where r_i (m) is the tree crown radius of tree *i*, and *V* is relascope angle determined by the measurement instrument and a design parameter that is constant for all trees. Thus, the inclusion probability $\pi_{i,HLS}$ can be expressed with replacing R_i as:

$$\pi_{i,HLS} = \frac{cd_i \times l}{10^4 A \tan\left(\frac{V}{2}\right)} \tag{7}$$

Using HLS, the HT estimator of AGB for a single sampling unit, j is:

$$\hat{\tau}_{AGB,HLS,j} = \frac{10^4 A \tan\left(\frac{V}{2}\right)}{l} \sum_{i=1}^m \frac{AGB_i}{cd_i} \qquad (8)$$

and the estimator of N for a single sampling unit, j is:

$$\hat{\tau}_{N,HLS,j} = \frac{10^4 A \tan{(V/2)}}{l} \sum_{i=1}^{m} \frac{1}{cd_i}$$
(9)

In the case of HLS, the estimator of the population total in a sample of *n* lines is:

$$\hat{\tau}_{HLS} = \frac{1}{n} \sum_{j=1}^{n} \hat{\tau}_{HLS,j} \tag{10}$$

For both attributes ABG and N the average per hectare can be estimated with

$$\frac{\hat{\tau}_{AGB}(or\,\hat{\tau}_N)}{A}.$$

In the study, we predicted tree-level biomass using the following equation fitted by Iranmanesh *et al.* (2014). The equation is based solely on the tree-level crown diameter and is defined as

$AGB = 0.881 \times cd^{3.228} \tag{11}$

where *cd* is the tree crown diameter (m). The authors developed Equation 11 using 30 sampled *Quercus brantii* trees. They found a strong and nonlinear relationship ($R^2 = 0.97$) between above ground biomass (*AGB*) and tree crown diameter (*cd*). The study area had a mean annual rainfall sum of 567 mm and a mean annual temperature of 15.5 °C.

2.3 Time study

The study recorded the time required in seconds per length unit for data acquisition, including measuring the diameter of the crown projected on the ground of sampled trees using a standard tape and walking to the sample trees. The work was carried out by a three-person crew.

2.4 precision assessment

As both inventory techniques offer design-unbiased estimates for the population total *N* and *AGB*, the comparison between the two techniques was based on the smallest cost-variance. The variability between line transects was used to characterize the sampling variability on the total estimates. The estimator of variance of the population total, $\hat{\tau}_{\gamma}$ is defined as (Thompson 2002):

$$\hat{V}(\hat{\tau}_{y}) = \frac{1}{n(n-1)} \sum_{j=1}^{n} (\hat{\tau}_{y,j} - \hat{\tau}_{y})^{2}$$
(12)

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where $\hat{\tau}_{y,j}$ is individual estimate of the population total of a single transect, j and $\hat{\tau}_y$ is the average of the all *n* transects. The relative sampling error (SE %) for the population

total,
$$\hat{\tau}_y$$
 is calculated as $\frac{\sqrt{\hat{v}(\hat{\tau}_y)}}{\hat{\tau}_y} \times 100$.

It should be noted that the sample size for all combinations of three relascope angles and three line transect lengths was the same (*i.e.*, 30 line transects).

2.5 Efficiency evaluation

The relative efficiency (RE) method is a commonly used approach to assess the efficiency of different forest inventory techniques (Husch, 1963), where both costs (time) and sampling error are considered. By this method, a forest inventory technique (here LIS), serves as the reference. RE can be computed by:

$$RE = \frac{\% SE_{HLS} \times T_{HLS}}{\% SE_{LIS} \times T_{LIS}}$$
(13)

where $\%SE_{HLS}$ and $\%SE_{LIS}$ are the relative sampling errors of *HLS* and *LIS*, respectively. T_{HLS} and T_{LIS} are the total time needed for inventory techniques *HLS* and *LIS*, respectively. When RE < 1, *HLS* is more efficient than *LIS*; when E > 1, the converse is true.

Standard statistical procedures were employed to analysis the data. Specifically, a one-way ANOVA was used to test for significant differences between the inventory techniques sued to estimate *N* and *AGB*. Additionally, a multiple regression analysis was conducted to examine the combined effect of predictor variables-line length and relascope angle-on response variable SE %.

3 Results

Figure 4 shows the estimates of the total *N* and *AGB* obtained using inventory techniques tested. The error bars represent confidence intervals. Although *HLS* and *LIS* produced different estimates, the difference was not statistically significant at a 90% confidence level (p=0.874 and p=0.876 for *N* and *AGB*, respectively), as shown in Tables A1 and A2. This result can be expected, since both inventory techniques are design-unbiased. In most cases, longer line transects resulted in smaller confidence intervals.



Figure 4: Estimates of the total N (top) and AGB (bottom) using LIS and HLS. Inclusion angles (V_{1r}, V_{2r}, V_{3}) and three line transect lengths. The error bars indicate the range within which we are 90% confident that the true value lies. This was determined using data from 30 line transects. The figures represent the total estimates for a 10 hectare area.

Abbildung 4: Schätzungen von N (oben) und AGB (unten) unter Verwendung von LIS und HLS. Einbeziehungswinkel (V₁, V₂, V₃) und drei Linientransektlängen. Fehlerbalken repräsentieren die Konfidenzintervalle und die Ergebnisse wurden aus einer Stichprobengröße von 30 Linientransekten gewonnen. Die Zahlen repräsentieren die Gesamtschätzungen für eine Fläche von 10 Hektar. Figure 5 displays the relative sampling errors (SE%) for *N* and *AGB* using two inventory techniques (LIS and HLS), three line transect lengths, and three relascope angles. In general, HLS is more precise than LIS. This result is expected since HLS covers a larger stand area than a comparable LIS, increasing the probability of sampling more trees. Additionally, in most cases, longer line transects result in lower relative sampling errors (SE%).



Figure 5: Relative sampling error (SE%) of HLS with three inclusion angles (V_1 , V_2 , V_3) compared to LIS for N (top) and AGB (bottom).

Abbildung 5: Relativer Stichprobenfehler (SE%) von HLS mit drei Einbeziehungswinkeln (V_1, V_2, V_3) im Vergleich zu LIS für N (oben) und AGB (unten).

The results of the multiple regression analysis, shown in Figure 6, indicate a negative relationship between the relative sampling error (SE%) and line transect length. This implies that an increase in the length of the line transect is associated with a decrease in the SE%. Conversely, there is a positive relationship between SE% and relascope angle, which means that an increase in the relascope angle is associated with an increase in the SE%.



Figure 6: The relationship between SE% and relascope angle and line length for both N (top) and AGB (bottom).

Abbildung 6: Die Beziehung zwischen SE% und Relaskopwinkel und Linienlänge für N (oben) und AGB (unten).

Table 2 provides the average time (in seconds per meter) for two inventory techniques and three relascope angles. It is important to note that the walking costs between sampling lines were not included.

Table 2: The average time needed to measure one meter of transect length for two inventory techniques and three different relascope angles, V (°).

Tabelle 2: Die durchschnittlich nötige Zeit, um einen Meter Transektlänge zu messen für zwei Inventurtechniken und drei verschiedene Relaskopwinkel, V (°).

	LIS	HLS		
		V ₁ (36.8°)	V ₂ (53.1°)	V ₃ (67.4°)
time needed (s/m)	8	25	15	11

Table 3 presents the relative efficiency (RE%) of three line transect lengths and three relascope angles. In some cases, the RE is less than or equal to 1. This indicates that in these cases, HLS is superior to LIS.

Table 3: Relative efficiency (RE) of three transect lengths and three relascope angles. In bold print, HLS is superior or equivalent to LIS in terms of cost-%SE.

Tabelle 3: Relative Effizienz (RE) von drei Linienlängen und drei Relaskopwinkeln. Fettgedruckte Zahlen zeigen, dass HLS überlegen oder gleichwertig ist zu LIS in Bezug auf Kosten-%SE.

		HLS	
Transect length (m)	\mathbf{V}_1	V_2	V_3
20	2.0	1.5	1.2
40	1.8	0.9	1.0
80	1.6	0.6	1.2

4 Discussion

Monitoring forest change and forest degradation is important in sustainable development and management of forest landscapes (Li *et al.* 2021). Therefore, efficient collection of forest information will play a significant role in improving the management of forest landscape (Moradi *et al.* 2022).

4.1 Estimation of N and AGB

The application of horizontal line sampling (HLS) in coppice forests, particularly in combination with crown relascope, is novel. As a result, there was no prior know-ledge of an appropriate crown basal area factor (CBAF) and its impact on estimates. CBAF was determined by practical convenience. Previous studies have shown that estimates of forest attributes are highly related to basal area factor (BAF) when using horizontal point sampling (HPS) (Brooks 2006; Lindemuth 2007). Our results show that there is no significant difference among the three CBAFs tested in estimating the total number of trees (*N*) and above-ground biomass (*AGB*). Thus, choosing much larger or smaller CBAFs may lead to significant estimates of *N* and *AGB*.

In this study, it is impossible to determine the most accurate inventory technique due to the lack of a reference value for *N* and *AGB*. Further simulation studies are needed to determine the most accurate inventory technique in coppice forests. Under a simulation study, it is also possible to test very large or small CBAFs.

4.2 Statistical properties

In general, line intersect sampling (LIS) resulted in higher variance, which was expected as the inclusion zone for trees in LIS – the "imaginary rectangle" – is smaller than in horizontal line sampling (HLS). Our results indicate that, in most cases, HLS is more efficient than LIS in terms of relative sampling error (SE %). This finding is consistent with Stenberg *et al.* (2008), where a Crown Relascope (CrR) was compared to LIS for estimating crown cover area in boreal forests. However, the authors combined CrR with horizontal point sampling (HPS) and did not consider costs.

Although horizontal line sampling (HLS) with V_1 resulted in the smallest sampling error for all three line lengths and for both the total number of trees (*N*) and aboveground biomass (*AGB*) (Fig. 5), it is not cost-effective. This is because many trees are sampled, making it time-consuming to use V_1 . One way to overcome this drawback – the walking time to sampled trees – is to use ground-based horizontal laser scanning to measure tree crown diameter. As our results show, HLS is superior to line intersect sampling (LIS) in terms of variance (Fig. 5). However, in terms of cost-efficiency, HLS is not effective in some cases because it is more time-consuming than LIS (Table 3).

4.3 Relationship between sampling error and line length and relascope angle

Our results indicate that both relascope angle and line length impact the relative sampling error (SE %). Rice *et al.* (2014) also found that for a given line length, a larger

relascope angle can produce a higher sampling error. Our results suggest that longer line transects and/or smaller relascope angles lead to better precision estimates. However, Schreuder *et al.* (1987) found in a simulation study that longer lines with a large angle can be efficient, which they attributed to dense undergrowth in the study area - a factor not present in our study. Previous studies have shown that HLS is equivalent or superior to HPS in various respects (*e.g.*, Ríos *et al.* 2000; Schreuder *et al.* 1987). Kenning *et al.* (2005) found that HLS was more efficient in sampling basal area compared to fixed area sampling. However, no comparison study has been conducted between HLS and LIS, making direct comparison of results difficult.

A wide-angle relascope was used by Cooper (1957, 1963) and Stenberg et al. (2008) to estimate the number of shrubs (*N*) and canopy cover area (*CCA*) in shrubland and boreal forest stands, but the authors applied HPS. They found that HPS was also superior to LIS. Using HPS, *CCA* can be estimated simply by counting sampled trees and multiplying by a constant expansion factor – the crown basal area factor. However, to estimate other forest attributes such as above-ground biomass (*AGB*) and *N*, the tree crown diameter of sampled trees must be measured.

4.4 Caveats and shortcomings

There are several sources of bias when using Horizontal Line Sampling (HLS): (a) deviations from the line transect when walking along it; (b) not operating the relascope horizontally; (c) overlapping of tree crowns; and (d) non-circularity of tree crowns. As Stenberg *et al.* (2008) concluded, CrR appears to be effective and applicable in forest stands where there is no overlapping of tree crowns. Fortunately, this is the case in our study area – a low-density, open coppice forest. Ståhl (1997) also found that the relative superiority of HLS was larger in populations where the intensity of population elements was low. The last source of bias was not included in this study, but it would be an interesting research topic to explore the extent of bias. For this purpose, a true value is needed.

4.5 A vision for the future of HLS and crown relascope research

In this study, line transects were established randomly. However, Gregoire and Valentine (2008) describe a systematic alternative for deriving element inclusion probabilities and corresponding estimators. Under a systematic design, the probability of including a tree depends on the width of the inclusion area for the tree in a direction perpendicular to the sample line. One advantage of a systematic sampling design is that it allows for the estimation of total without the need for the total area of the study tract (Lynch, 2006). In this study, the sample size is fixed (*i.e.*, 30 line transects). However, it is well-known that sample size can substantially impact the precision of estimates. Woldendorp *et al.* (2004) and Ramezani and Holm (2010) demonstrated in simulation surveys how sample size and line transect length can influence the precision of estimates. It would be interesting to conduct a similar survey for CrR and HLS, considering a combination of different sample sizes, line transect lengths, and relascope angles. It would also be interesting to compare crown relascope to fixed-area sampling, a widely used forest inventory technique.

5 Conclusions

In this study, we explored a new application of Horizontal Line Sampling (HLS) in combination with CrR to estimate attributes of coppice forests (CFs). Our suggested combination is easy to implement in the field and, in some cases, more efficient than Line Intersect Sampling (LIS) for estimating forest attributes in CFs. While this study focused on standing trees, we speculate that HLS and CrR may also be useful for inventorying other sparse populations such as downed wood in CFs.

Both relascope angle and line transect length impact the performance of HLS. The technique used in our research may be replicated in other CFs with similar characteristics and stand structures. Our study provides evidence that this technique can also support forest degradation monitoring.

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Appendix

Table A1: One-way ANOVA to test whether there is a significant difference between inventory techniques in the estimation of N.

Tabelle A1: Einfaktorielle ANOVA, um zu testen, ob es einen signifikanten Unterschied zwischen Inventurtechniken bei der Schätzung von N.

Source of Variation	SS	df	MS	F	P-value
Between inventory techniques	421125.4	11	38284.1	0.541	0.874
Within inventory techniques	24606196.8	348	70707.4		
Total	25027322.2	359			

Table A2: One-way ANOVA to test whether there is a significant difference between inventory techniques in the estimation of AGB.

Tabelle A2: Einfaktorielle ANOVA, um zu testen, ob es einen signifikanten Unterschied zwischen Inventurtechniken bei der Schätzung von AGB.

Source of Variation	SS	df	MS	F	P-value
Between inventory techniques	1.01356E+11	11	9214175460	0.539	0.876
Within inventory techniques	5.94679E+12	348	17088463351		
Total	6.04814E+12	359			

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Ectomycorrhizal Symbionts of Scots Pine and Silver Birch Forest Ecosystems in the Natural Reserve Ertis Ormany in Kazakhstan

Ektomykorrhiza-Symbionten in Waldökosystemen der Waldkiefer und Hängebirke im Naturschutzgebiet Ertis Ormany in Kasachstan

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- Keywords:Betula pendula, Pinus sylvestris, fungal diversity, symbiosis,
symbiotic relationships, species saturation coefficient,
Agaricales, Russulales, Boletales
- Schlüsselbegriffe: Betula pendula, Pinus sylvestris, Pilzdiversität, Symbiose, symbiotische Beziehungen, Artensättigungskoeffizient, Agaricales, Russulales, Boletales

Abstract

Symbiotic relationships maintain the balance of forest ecosystems, with ectomycorrhizal (EcM) symbiosis being one of the most significant. EcM symbiosis in forests dominated by Scots pine (*Pinus sylvestris* L.) and silver birch (*Betula pendula* Roth.) has been often observed, but remain understudied in parts of world. This study was conducted in the State Forest Natural Reserve "Ertis Ormany" in Kazakhstan. The aim of this study was to identify EcM symbionts on Scots pine and silver birch by analyzing ribosomal DNA sequences isolated from hyphal endings. Our results show that among all EcM symbionts the order *Agaricales* (13 species/28.8% of total species numer) had highest species diversity in Scots pine, followed by *Boletales* (9/20%) and *Russulales* (8/17.7%). For silver birch, the order *Russulales* (7/15.5%) had highest species diversity, followed by *Agaricales* (5/11.1%), and *Boletales* (3/6.7%). A total of 15

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symbiont families were identified: *Agaricaceae* (2 species/4,4% of the total number of species identified in the study area), *Amanitaceae* (4/8.9%), *Boletacea* (5/11.1%), *Cortinariaceae* (3/6.7%), *Gloeophyllaceae* (1/2.2%), *Inocybaceae* (1/2.2%), *Lycoperdaceae* (1/2.2%), *Lycophyllaceae* (2/4.4%), *Paxillaceae* (1/2.2%), *Polyporaceae* (3/6.7%), *Psathyrellaceae* (2/4.4%), *Pseudoclitocybaceae* (2/4.4%), *Russulaceae* (8/17.8%), *Suillaceae* (6/13.3%), and *Tricholomataceae* (4/8.9). A higher symbiotic association with EcM was found in Scots pine (66.5%) compared to silver birch (33.3%).

Zusammenfassung

Symbiotische Beziehungen sind wichtig für die Stabilität von Waldökosystemen, wobei Ektomykorrhiza (EcM)-Symbiose einen besonderen Stellenwert hat. Die EcM-Symbiose an Waldkiefer (Pinus sylvestris L.) und Hängebirke (Betula pendula Roth.) wurde recht häufig beobachtet, ist aber in vielen Teilen der Welt wenig erforscht. Das Forschungsgebiet war das Naturschutzgebiet "Ertis Ormany" in Kasachstan. Unsere Ergebnisse zeigen, dass unter allen Symbionten der Waldkiefer die Ordnung Agaricales (13 Arten/28,8 % der gesamten Artenzahl) und Boletales (9 Arten/20 %) die höchste Artenvielfalt aufwiesen, gefolgt von Russulales (8/17,7 %). Bei der Hängebirke waren die Ordnungen Agaricales (5/11,1 %), Boletales (3/6,7 %) und Russulales (7/15,5 %) führend. Insgesamt wurden hier bei den Symbionten der Waldkiefer und Hängebirke 16 Familien identifiziert: Agaricaceae (2/4,4%), Amanitaceae (4/8,9%), Boletacea (5/11,1%), Cortinariaceae (3/6,7%), Gloeophyllaceae (1/2,2%), Inocybaceae (1/2,2 %), Lycoperdaceae (1/2,2 %), Lyophyllaceae (2/4,4 %), Paxillaceae (1/2,2 %), Polyporaceae (3/6,7%), Psathyrellaceae (2/4,4%), Pseudoclitocybaceae (2/4,4%), Russulaceae (8/17,8%), Suillaceae (6/13,3%) und Tricholomataceae (4/8,9%). Es gibt einen Trend zu einem höheren Koeffizienten der Artensättigung mit EcM-Symbionten bei der Waldkiefer (66,5 %) im Vergleich zu der Hängebirke (33,3 %).

1 Introduction

Symbiotic relationships are one of the most important components for maintaining the balance and stability of forest ecosystems (Courty *et al.*, 2010). A common form of symbiotic relationship in forest ecosystems is the ectomycorrhizal (EcM) symbiosis. It is well known that the diversity and structure of EcM symbiosis are influenced by a number of biotic and abiotic factors such as host tree genotype, mycosymbiont interaction strategy, soil characteristics, availability of nutrients, the activity of pathogens, and quality and quantity of soil organic matter (Vaishlya *et al.*, 2019). The impacts of climate change may alter belowground fungal communities (Pickles *et al.*, 2012), so that they may become a limiting factor in tree migration. Furthermore, knowledge of the linkages among soil fungal diversity, community structure, and environmental

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factors is critical to understand the organization and stability of forest ecosystems (Simard and Austin, 2010; Karst *et al.*, 2014).

The EcM symbiosis of Scots pine (*Pinus sylvestris* L.) and silver birch (*Betula pendula* Roth) is quite often observed in forest ecosystems. Ectomycorrhiza is formed by about 5-6 thousand plant species (Voronina, 2006; 2008), almost exclusively woody or shrub species from Gymnosperms (family *Pinaceae*, *Cupressaceae*) and Angiosperms (18 families, most important are *Fagaceae*, *Betulaceae*, *Salixaceae*, *Myrtaceae*, *Aceraceae*, *Tiliaceae*, *Ulmaceae*) (Molina *et al.*, 1992).

EcM fungi are obligate mutualistic symbionts and hence rely entirely on carbon supply from host plants (Smith and Read, 2008). While they are abundant in root systems of herbaceous plants (Hiiesalu et al., 2014), they are also associated with various tree species (Liu et al., 2015). EcM fungi play a major role in forest community dynamics (Smith and Read, 2008) as they promote the dominance of specific plant families e.g., Pinaceae, Fagaceae, Betulaceae, and Dipterocarpaceae (Tedersoo and Smith, 2013). Ectomycorrhizae are found in the upper layer of the soil, which mostly contains humus, rather than the lower layers which contain mineral-rich substances (Dogmus Lehtijarvi, 2007). These fungi improve plant nutrition in exchange for carbohydrates (Smith and Read, 1997; Sebastiana et al., 2018). They have an important role in the intake of Zn, Cu, Mn, Fe, Ca, K, N, and particularly P, due to slow uptake from the soil. Moreover, mycorrhiza increase water use efficiency, as well as improve soil structure and protect against soil erosion (Dogmus Lehtijarvi, 2007). Previous studies stated that the use of mycorrhizal seedlings in afforestation, rehabilitation and restoration works in marginal sites can significantly increase the success of plantations (Arocena and Glowa, 2006; Qiang-Sheng and Ren-Xue, 2006; Bennett et al., 2017; Kharuk et al., 2019). This subject is important in marginal sites with extreme ecological conditions and higher climate change influence (Barbati et al., 2018). The environmental conditions on poor sites favour numerous harmful biotic factors including pathogens (Haavik et al., 2015).

Studies of the ecology and physiology of EcM have been concentrated mainly in Europe, North America, and Australia (Smith and Read, 1997; 2008; Read, 1999; Finlay, 2005; Polenov, 2013). In the Republic of Kazakhstan, first studies of mycobiota and EcM macromycetes were carried out by Nam (1998), Abiev *et al.* (2000), and Abiev (2015). Applied aspects of the mycorrhization were investigated by Meshkov (2010). He obtained four types of macromycetes from the investigated culture and developed a technology for their scaling and application in inocculated compost for reforestation in Zailiysky Alatau (Meshkov *et al.*, 2009; Meshkov 2010). However, some researchers emphasize that for optimal development of trees, specialized strains of macromycetes fungi forming EcM are needed (Alvarez and Trappe, 1983; Valdes, 1986). Especially, coniferous seedlings are not able to achieve adequate growth, if they are excluded from mycorrhizal occurrence (Kais *et al.*, 1981; Alvarez and Trappe, 1983; Valdes, 1986).

Preservation of environmentally favorable living conditions in various regions of the world directly depends on the rational and careful use of forest resources. Forest ecosystems of Central and Northeast Kazakhstan are one of the most important components of the Earth's biosphere, contributing to the ecological balance on the entire planet (Sarsekova *et al.*, 2021). In this context, Godbold *et al.* (2006) draw attention that an increase in mycorrhizal hyphal biomass and turnover would increase carbon sequestration in terrestrial ecosystems. The assessment of EcM symbionts of forest trees in Kazakhstan's forest ecosystems could help to improve regeneration, afforestation, rehabilitation, and restoration efforts in this region which is affected by climate change and extreme ecological conditions. The main objectives of this study were:

- to establish basic information about EcM associated with Silver birch and Scots pine forests in the protected area "Ertis Ormany" in Kazakhstan, using molecular methods isolating DNA from hyphal endings of the EcM and subsequent sequencing of ribosomal DNA,
- (2) to determine whether there are differences in the quantity of mycorrhizal symbiosis and in the preference of forest tree species, and
- (3) to investigate if there are new undescribed ectomycoriza species in these silver birch and Scots pine forests.

2 Materials and Methods

2.1 Study area

The Republican State Institution State Forest Natural Reserve "Ertis Ormany" is located on the eastern part of the Pavlodar region on the right bank of the Irtysh river in Kazakhstan (Figure 1) (Northern latitude 51°23″ – 52° 15″, Eastern longitude 78° 01″ – 79° 21″). It is within the borders of Shcherbaktinsky district and Akkulinsky district (Anonymous, 2009) and covers an area of 277,961 hectares.



Figure 1: The natural distribution area of Pinus sylvestris L. and Betula pendula Roth. (Caudullo et al., 2018) and the location of the study area in Kazakhstan.

Abbildung 1: Das natürliche Verbreitungsgebiet von *Pinus sylvestris* L. und *Betula pendula* Roth. (Caudullo *et al.*, 2018) und die Lage des Untersuchungsgebiets in Kasachstan.



Figure 2: Satellite image map showing sampling areas of the study area.

Abbildung 2: Satellitenbild mit Position der Probenahmen im Untersuchungsgebiet.

Territories of the state nature reserves are divided into zones according to different types of protection and use:

- i) a protected area is a core zone intended for the long term conservation of genetic resources, biological diversity, ecological systems, and landscapes, having sufficient features to achieve such goals;
- ii) buffer zone a section of territory that is used for conducting environmentally oriented economic activities and sustainable reproduction of biological resources (Figure 2, 3) (Forest Code of the Kazakhstan Republic, 2003).

b c

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Figure 3: Sample areas of Scots pine and silver birch; a) \mathbb{N}^{0} 1 sample area of Scots pine in Shaldai forest; b) \mathbb{N}^{0} 6 Scots pine in Taybagarskoe forest; c) example of selected seedlings of Scots pine; d) \mathbb{N}^{0} 3 sample area of Silver birch in Taybagarskoe forest; e) \mathbb{N}^{0} 8 silver birch in Zavodskoe forest; f) example of soil blocks under crown of silver birch.

Abbildung 3: Untersuchungsflächen der Waldkiefer und der Hängebirke; a) Nr. 1 Untersuchungsgebiet der Waldkiefer im Shaldai-Wald; b) Nr. 6 Waldkiefer im Taybagarskoe-Wald; c) Beispiel eines ausgewählten Sämlings der Waldkiefer; d) Nr. 3 Untersuchungsgebiet mit Hängebirke im Pervomayskoe-Wald; e) Nr. 8 Versuchsfläche der Hängebirke im Zavodskoe-Wald; f) Beispiel eines Bodenblocks unter der Krone von Hängebirke.

2.2 Natural and climatic conditions of the study area

The climate in the study area is continental characterized by dry conditions due to dry and hot winds blowing from the Central Asian deserts during summer and winter. The area is open to cold air flows coming from the north. Winters are cold and last long (5.5 months) and summers are short and hot with low precipitation, with extreme temperature fluctuations between winter and summer (range 88 °C) and

between day and night (range 22 °C). The average annual air temperature is between +2.5 and +3 °C, average temperature of the coldest month (January) ranges between -17 and -19 °C and about +21 °C in the warmest month (July). The absolute minimum and maximum temperatures ever recorded at the Shaldai weather station were -49 °C and +41°C, respectively. The growing season lasts 137 days on average. In summer, relative air humidity (at 13:00) is about 40%, sometimes decreasing to 10% causing intensive transpiration of plants and a large loss of moisture from the soil. Mean annual precipitation sum ranges between 240 to 310 mm (Anonymous, 2015).

2.3 Methods

The "Ertis Ormany" reserve is covered by various forest ecosystems – pure and mixed forests with silver birch and Scots pine (Figure 1). Within this forests, seven permanent transects were established. In each transect, macromycetes were collected and inventoried every 10-15 days during the spring season from May to September in 2020-2022. A total of 110 samples were collected, 60 of these were soil samples and the other 50 samples were collected from the root systems of tree seedlings. Transects were established according to the methodology of Villarreal-Ruiz and Neri-Luna (2018). In total, 60 soil cores were taken about every 2 meters along each 125 m transect.

Collection and identification of the EcM fruit bodies was performed following standard methods, using available determinants and different web sources (http://www. indexfungorum.org and http://www.mycobank.org). Micromorphological studies were carried out using a light microscope ("Altami SM745-T", Russia) at a magnification of 400-1000. To identify the natural color of microstructures, samples were viewed in distilled water and in 3-5% KOH solution. Hyaline structures were stained with a 5% aqueous solution of safranin, and the presence or absence of amyloid and dextrinosis structures were determined using Meltzer reagent (Vaishlya *et al.*, 2012).

To determine the type of fungal symbiont of the EcM, the Agerer morphotyping method was used (Agerer, 1987; 1991; Agarer *et al.*, 2002). For this purpose, soil samples were selected within the projection of the crown of mature trees according to the method of concentric sampling (Vaishlya *et al.*, 2019). Before sampling, the upper layer of litter was removed. The seedlings were extracted from the soil without disturbing their root systems. In total, 210 root ends were examined, 113 for Scots pine and 97 for silver birch.

The samples were wrapped in aluminum foil and stored at a temperature of +4 °C. The roots were washed with running water and cut into segments of 3-5 cm. EcM endings were separated under a magnifying glass with tweezers and scissors. Morphotyping of ectomycorrhizae was performed using a binocular microscope "Alta-

mi SM745-T" with a camera Altami UCMOSO3100KPA and according to the DEEMY (http://deemy.de) system, based on the nature of branching, the color of the EcM termination, features of the mantle surface, the presence or absence of external mycelium and rhizomorphs. The selected EcM samples (113 for *Pinus sylvestris* and 97 for *Betula pendula*) were photographed and fixed in 70% ethanol for DNA isolation. DNA isolation from the sampled EcM hyphal endings was performed using a standard Quiagen kit based on the attached protocol (Vaishlya, 2017). ITS-1F and ITS-4B primers were used for amplification and sequencing of the ITS nuclear DNA site. Sequencing was performed on the ABI 3130 Genetic Analyzer (Applied Biosystems) with subsequent processing of the data obtained in the BioEdit Sequence Alignment Editor application. Identification of EcM fungi to generic and species taxa was performed using the M algorithm by comparison with reference sequences from the open GenBank database (http://www.ncbi.nlm.nih.gov/genbank/), taking into account the lower threshold of 97-98% established for ITS regions of ribosomal genes of fungal DNA (Köljalg *et al.*, 2013).

The nucleotide sequences were checked and manually edited using SeqScape V3.0 or Vector NTI Advance 11.5.1 software. Subsequently, they were compared with sequences deposited in GenBank using the BlastN search (Altschul *et al.*, 1990). The newly obtained ITS sequences were deposited in GenBank under unique accession numbers.

Of all the known DNA marker sequences, the International Fungal Barcoding Consortium has recognized the ITS region of ribosomal genes of nuclear DNA as the main marker in the molecular genetic method for fungi species identification (Schoch *et al.*, 2014). When determining the boundaries of the studied operational taxonomic units, we proceeded from the previously established lower cutoff value (treshold) = 97-98% for fungi' ITS (Köljal *et al.*, 2013).

3 Results and Discussion

The distribution of ectomycorrhizas (EcM species) according to tree species is given in table 1. Our results reveal, that among all the symbionts of Scots pine forests (Figure 4), the orders *Agaricales* (13 species/28.8%) and *Boletales* (9/20%) had the highest species diversity, followed by the order *Russulales* (8/17.7%) with a smaller number of species (Table 1).

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Figure 4: Photos of various ectomycorrhizae morphotypes of the root system of Scots pine and silver birch. *a*-Lyophyllum decastes (Fr.) Singer, b-Lactarius sp., c-Lactarius rimosellus Peck, d-Russula sp.1, e-Amanita sp., f-Suillus sp., g-Candolleomyces candolleanus (Fr.) D. Wächt. & A. Melzer, h-Psathyrella sp., i-Pseudosperma sp., j-Lycoperdon norvegicum Demoulin, k-Russula medullata Romagn, l-Tricholoma sp.

Abbildung 4: Fotos verschiedener Ektomykorrhizae-Morphotypen des Wurzelsystems von Waldkiefer und Hängebirke. a- Lyophyllum decastes (Fr.) Singer, b- Lactarius sp., c- Lactarius rimosellus Peck, d-Russula sp.1, e- Amanita sp., f- Suillus sp., g- Candolleomyces candolleanus (Fr.) D. Wächt. & A. Melzer, h- Psathyrella sp., i- Pseudosperma sp., j- Lycoperdon norvegicum Demoulin, k- Russula medullata Romagn, I- Tricholoma sp.
Table 1: Ectomycorrhizal symbionts identified from root endings and macromycetes of Pinus sylvestris and Betula pendula.

Tabelle 1: Ektomykorrhiza-Symbionten identifiziert aus Wurzelenden und Makromyceten von *Pinus sylvestris* und *Betula pendula*.

Genus/Species name of the EcM	Order/Family	Genbank number	The result of Blast (Genbank Unite)	Similarity, %	Tree species of the symbiotic relationship
1. Agaricus sp.	Agaricales, Agaricaceae	ON704909	Agaricus litoralis (Wakef. & A. Pearson) Pilát	98.98	Pinus sylvestris L.
2. Amanita pantherina (DC.) Krombh	Agaricales, Amanitaceae	ON704911	Amanita sp.	97.46	Pinus sylvestris L., Betula pendula Roth.
3. Amanita sp.	Agaricales, Amanitaceae	ON704908	Amanita pseudopantherina Zhu L. Yang ex Y.Y. Cui	98.84	Pinus sylvestris L., Betula pendula Roth.
4. Bonomyces sp.	Agaricales, Pseudoclitocybaceae	ON704912	Bonomyces sp.	99.85	Pinus sylvestris L.
5. Candolleomyces sp.	Agaricales, Psathyrellaceae	OP215188	Candolleomyces candolleanus (Fr.) D. Wächt. & A. Melzer	99.16	Pinus sylvestris L., Betula pendula Roth.
6. Cortinarius sp.	Agaricales, Cortinariaceae	ON704900	Cortinarius croceus (Schaeff.) Gray	99.67	Pinus sylvestris L.
7. Chlorophyllum sp.	Agaricales, Agaricaceae	ON704902	Chlorophyllum sp.	96.70	<i>Betula pendula</i> Roth.
8. Lactarius sp.	Russulales, Russulaceae	OP215189	<i>Lactarius</i> rimosellus Peck	100	<i>Betula pendula</i> Roth.
9. Lactarius sp.	Russulales, Russulaceae	ON704904	Lactarius sp.	99.82	<i>Betula pendula</i> Roth.
10. Lycoperdon sp.	Agaricales, Lycoperdaceae	ON704897	<i>Lycoperdon norvegicum</i> Demoulin	100.00	Pinus sylvestris L.
11. Lyophyllum sp.	Agaricales, Lyophyllaceae	ON704896	Lyophyllum decastes (Fr.) Singer	99.38	<i>Betula pendula</i> Roth.
12. Psathyrella sp.	Agaricales, Psathyrellaceae	ON704906	Psathyrella sp.	99.83	Pinus sylvestris L.

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13. Pseudosperma sp.	Agaricales, Inocybaceae	ON704906	Pseudosperma sp.	97.14	Pinus sylvestris L.
14. Russula sp. 1	Russulales, Russulaceae	ON704914	Russula medullata Romagn.	100.00	Betula pendula Roth
15. Russula sp. 2	Russulales, Russulaceae	OP215195	Russula sp.1	99.82	Betula pendula Roth
16. Russula sp. 3	Russulales, Russulaceae	OP215193	Russula sp.2	99.70	Betula pendula Roth
17. Russula sp. 4	Russulales, Russulaceae	ON704905	Russula sp.3	99.77	Betula pendula Roth
18. Russula sp. 5	Russulales, Russulaceae	ON704898	Russula sp.4	94.94	Betula pendula Roth
19. Suillus sp. 1	Boletales, Suillaceae	ON704899	Suillus brunnescens A.H. Sm. & Thiers	98.47	Pinus sylvestris L.
20. Suillus sp. 2	Boletales, Suillaceae	OP215196	Suillus sp.	97.28	Pinus sylvestris L.
21.Tricholoma sp. 1	Agaricales, Tricholomataceae	ON704910	Tricholoma robustum (Alb. & Schwein.) Ricken	99.87	Pinus sylvestris L.

A similar ratio of the number of species in the orders was found in Russia, for example, for the Stolby Nature Reserve of the Krasnoyarsk Territory (Kosheleva, 2007), the Urals (Fefelov, 2005) and the Lower Volga Region (Zemlyanskaya, 2003). Regional differences are manifested only at the level of species saturation of genera and families. According to recent studies, EcM has a close functional connection with the formation of the structure, diversity, and stability of plant communities (Püschel et al., 2007; Lambers et al., 2008; Veselkin, 2012a; 2012b). Different forest types and favorable environmental conditions in forests promote high EcM species richness in top soil presumably with both high functional diversity and phylogenetic redundancy while stressful environmental conditions lead to lower species richness and functional redundancy (Pena et al., 2017). The ratio of symbionts in EcM may depend on factors such as the functional age state and the phenological state of the tree (Veselkin, 2013). Bahram et al. (2012) emphasized that EcM fungal diversity became lower at the elevated sites and was correlated with the soil temperature. In addition, Sizonenko and Dubrovskiy (2018) stated that a decline in the ectomycorrhiza linear dimensions accompanied by a compensatory growth of the ectomycorrhiza density with the raised elevation and the deterioration of environmental conditions. The composition of EcM communities is affected by large-scale patterns such as stand age, climatic conditions, soil-fauna and spore dispersal. The relationship between mycorrhizal fungi and soil acidity has long been discussed in the literature (Kalliokoski *et al.*, 2010; Pena *et al.*, 2017). EcM fungi are an important source of organic acids in soil (Griffiths *et al.*, 2009) and affect the pH of the rhizosphere (Smith and Read, 2008). On the other hand, soil conditions affect species diversity of EcM fungi as demonstrated by the dramatic decrease in species richness along a gradient of increasing nitrogen deposition (Lilleskov *et al.*, 2002). Altering nitrogen conditions in soil affects the outcome of species interactions possibly disadvantaging species adapted to the pre-change conditions while favoring generalists (Koide *et al.*, 2005). Mycorrhiza has been noted to decrease the pH in the rhizosphere, because of the selective absorption of ammonium NH+ ions and the release of H+ ions (Eremin and Popova, 2016). Smith *et al.* (2003) stated that the low effectiveness of root and mycorrhizal functions in the cold soils is due to insufficient mineral elements supply.

Otgonsuren et al. (2020) detected 24 different ectomycorrhizal morphotypes associated with Pinus sylvestris and Pinus sibirica, and 18 morphotypes were identified to taxa in forests in Mongolia. The two Pinus species had dissimilar ectomycorrhizal communities, and only 2 ectomycorrhizal fungal taxa were common to both species. In another survey conducted by Sarsekova et al. (2020), 30 ectomycorrhizas belonging to Agaricomycetes were identified on the right bank of the Irtysh River in a mixed forest of Pinus sylvestris, Picea obovata and Betula pendula trees of northern and central Kazakhstan. In their study, the distribution of 30 species into families was as follows; Suillaceae (8 species), Russulaceae (7), Cortinariaceae (4), Boletaceae (3), Tricholomataceae (2), Amanitaceae (1), Cantharellaceae (1), Gomphaceae (1), Gomphidiaceae (1), Paxillaceae (1), and Bankeraceae (1). The genus with the highest number of recorded species was Suillus (8). At the same time Sarsekova et al. (2020)'s study, 17 ectomycorrhizas were recorded under Pinus sylvestris, 8 under Betula pendula, 6 under Populus tremula, 1 under Picea obovata, 1 under Quercus robur, 1 under Salix sp., and 1 under Pinus densiflora. It should be noted that some species such as Paxillus involutus. Russula undulata, Cortinarius sp. and Cantharellus cibarius formed a symbiotic relationship with both coniferous and broadleaved tree species (Sarsekova et al., 2020). According to Garcia et al. (2016) the basidiomycete Rhizopogon spp. is typical in the root systems of the genus Pinus which is represented by the most species in the dominant regions of the Pinaceae family in the Pacific northwest of the USA. However, the symbiotic relationship of Scots pine and Rhizopogon sp. has not been found within the scope of our study. Massicotte et al. (1992) stated that the ascomycete Cenococcum graniforme (Sow.) Fred and Winge is the most common ectomycorrhizal fungus in the World, surprisingly this species has not been found in our study.

In the present study, five ectomycorrhiza families were identified in the silver birch stands: (5 species/25% of the total number of species identified in the studied area), *Amanitaceae* (3/6.7%), *Boletaceae* (3/6.7%), *Lycoperdaceae* (1/2.2%), *Paxillaceae* (1/2.2%), and *Russulaceae* (3/6.7%) (Tab. 1).

The list of families (with number and proportion) for all recorded species in the investigated area is as follows: *Agaricaceae* (2 species 2/4.4%), *Amanitaceae* (4/8.9%), *Boletacea* (5/11.1%), *Cortinariaceae* (3/6.7%), *Gloeophyllaceae* (1/2.2%), *Inocybaceae* (1/2.2%), *Lycoperdaceae* (1/2.2%), *Lyophyllaceae* (2/4.4%), *Paxillaceae* (1/2.2%), *Polyporaceae* (3/6.7%), *Psathyrellaceae* (2/4.4%), *Pseudoclitocybaceae* (2/4.4%), *Russulaceae* (8/17.8%), *Suillaceae* (6/13.3%), and *Tricholomataceae* (4/8.9). The coefficient of species saturation orders is: *Agaricales* 28.8, *Boletales* 20, *Russulales* 17.7. They included 45 of all species defined and identified with DNA 28 species in the study area (Figure 4). The remaining 16 genera included 6.2% of all identified species.

The average species saturation of pine forests is 10.0, which is lower than the average species saturation of 18.6 in the Republic of Kazakhstan (Novozhilov, 2005). This trend is explained by the locality of the territory and the absence of many types of substrates that have been studied in other regions of the Republic of Kazakhstan. For example, those that are often found in the steppe and desert (excrement of herbivorous animals, remnants of succulents, wormwood Artemísia absínthium L.). The leading families are those families where the number of species exceeds the average species saturation of families in the study area. As a result of this study, fundamental differences were found between the species saturation of EcM symbionts of Scots pine and Silver birch forests. In addition, Shi et al. (2016) stated that there are still critical gaps remaining in our understanding of biogeographic patterns of mycorrhizal associations. Further, our limited knowledge of the anthropogenic factors responsible for shifting plant-mycorrhizal distributions has hindered the efforts to predict ecosystem feedbacks to climate change. Mäkelä et al. (2022) suggest that EcM can use a significant proportion of the carbon assimilated by vegetation and hence be an important driver of the decline in carbon use efficiency at higher latitudes. Within this context, Godbold et al. (2006) emphasized that the turnover of the mycorrhizal external mycelium may be a fundamental mechanism for the transfer of root-derived C to soil organic material.

In total, 110 samples were collected during field work. Seventeen species were identified using classical methods via macromycete determinants, including EcM list of De Roman *et al.* (2005), and 28 were identified using DNA sequencing analysis (Figure 5). In the 60 soil samples 22 morphotypes of ectomycorrhizal fungal taxa associated with Scots pine and silver birch were determined. For the identify, a list from the study by De Roman *et al.* (2005), Tedersoo (2007), electronic resources (Ilyina, 2019; Polenov, 2013), as well as the Index Fungorum database and MycoBank (Robert *et al.*, 2020) were used.



Figure 5: The number of occurrences of taxa of EcM fungi in soil samples of Scots pine and silver birch.

Abbildung 5: Die Anzahl der Vorkommen von Taxa von EcM-Pilzen in Bodenproben von Waldkiefer und Hängebirke.

4 Conclusion

Five EcM families (*Psathyrellaceae, Russulaceae, Amanitaceae, Tricholomataceae,* and *Boletaceae*) were predominant in the Scots pine and silver birch forests studied here, with in total 11 species. These species are *Candolleomyces pseudocandolleanus* (A.H. Sm.) D. Wächt. & A. Melzer, *Amanita muscaria* (L.) Lam., *A. pantherina* (DC.) Krom., *Russula medullata* Romagn, *Suillus bovinus* (L.) Roussel, *Tricholoma robustum* (Alb. & Schwein.) Ricken, *Russula* sp.1, *Leccinum scabrum* (Bull.) Gray, L. *versipelle* (Fr. & Hök) Snell, *Psathyrella* sp., *Cortinarius croceus* (Schaeff.) Gray. In total, we managed to isolate the DNA of only 28 fungi species and the remaining species were determined by classical method using macromycetes.

In this study, a higher symbiotic association with EcM was found in Scots pine forests compared to Silver birch forests. A similar trend is observed not only in this study area but also in other parts of Kazakhstan. Our study allow us to conclude that the species and quantitative composition of Scots pine and silver birch forests vary considerably. The findings of this study are important for restoring unforested areas. In this context, EcM may be used as a major performance-enhancing tool in afforestation practices in the Irtysh River basin, where extreme ecological conditions and climate change effects prevail.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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Use of GUIDOS to analyze fragmentation features and test corridor creation for a fragmented forest ecosystem in Northern-Central Turkey

Verwendung von GUIDOS zur Analyse von Fragmentierungsmerkmalen und zur Prüfung der Korridorbildung in einem fragmentierten Waldökosystem im nördlichen Zentrum der Türkei

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Schlüsselbegriffe:	Waldfragmentierung, GUIDOS	Aufforstung,	Landschaftsökologie,		

Abstract

Fragmentation of forest habitats is an environmental challenge that is often caused by unsuitable forest management applications and socioeconomic reasons. Habitat fragmentation is considered as one of the most important causes of biodiversity loss. Establishing tree corridors using silvicultural approaches has shown to be beneficial for ecosystem restoration efforts. Various fragmentation and corridor analyses supported by remote sensing, geographic information systems and mapping of habitats in the field are now frequently used to determine the fragmentation status of forests and to create the most suitable corridors. In this study, the fragmentation status of all forest habitats within the boundaries of Çankırı State Forest Enterprise (ÇSFE) located in the north-central region of Turkey was determined, and opportunities to improve connections between fragmented forest clusters with the corridor approach were explored. We used the Graphical User Interface for the Description of Image Objects and their Shapes (GUIDOS) Version 3.0 for fragmentation analysis and corridor formation tests, structural assessment, network, and component-connection analyses.

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We found that forest area increased by +23% within the last 30 years, but habitat fragmentation was identified in nearly all forest textures. One of the reasons were afforestation activities, that did not consider the principles of landscape ecology. If the recommendations proposed here are implemented, will help ensure long-term sustainable management in light of landscape ecology principles for forests with different fragmentation conditions, not just in the study area but also in different regions of Turkey with similar environmental conditions.

Zusammenfassung

Die Fragmentierung von Lebensräumen in Wäldern ist eine ökologische Herausforderung, die oft durch ungeeignete Waldbewirtschaftung und sozioökonomische Gründe verursacht wird. Fragmentierung von Lebensräumen gilt als eine der Hauptursachen für den Rückgang der biologischen Vielfalt. Schaffung von Baumkorridoren mittels geeigneter waldbaulicher Techniken kann bei der Renaturierung von Ökosystemen helfen. Verschiedenste Fragmentierungs- und Korridoranalysen, unterstützt durch Fernerkundung, Geoinformationssysteme und Feldkartierung von Lebensräumen, werden häufig eingesetzt, um den Fragmentierungsstatus von Wäldern zu quantifizieren und die am besten geeigneten Korridore zu schaffen. In dieser Studie wurde der Fragmentierungsstatus aller Waldlebensräume innerhalb der Grenzen des Cankırı State Forest Enterprise (CSFE) im nördlichen Zentrum der Türkei ermittelt und Möglichkeiten zur Stärkung der Konnektivität fragmentierter Waldgebiete getestet. Wir verwendeten das Graphical User Interface for the Description of Image Objects and their Shapes (GUIDOS) Version 3.0 für die Fragmentierungsanalyse, die Korridorbildungstests sowie die Strukturbewertung, Netzwerk- und Komponentenverbindungsanalysen. Die Waldfläche hat in den letzten 30 Jahren um etwa +23 % zugenommen, dennoch wurde in fast allen Waldstrukturen eine Fragmentierung der Lebensräume festgestellt. Einer der Hauptgründe dafür waren eine fehlerhafte Aufforstung, bei der die Grundsätze der Landschaftsökologie nicht beachtet wurden. Eine Umsetzung der hier vorgeschlagenen Empfehlungen kann helfen, eine langfristige, nachhaltige Bewirtschaftung unter Berücksichtigung der Grundsätze der Landschaftsökologie für Wälder mit unterschiedlichen Fragmentierungsbedingungen sicherzustellen, nicht nur im Untersuchungsgebiet, sondern auch in verschiedenen Regionen der Türkei mit ähnlichen Umweltbedingungen.

1 Introduction

Habitat fragmentation can be defined as transforming the original habitat into a different habitat matrix comprising small isolated patches (Wilcove *et al.*, 1986). Many studies proposed that habitat fragmentation is one of the important leading causes of biodiversity loss (Bascompte *et al.*, 2002; Fahrig, 2002; Hanski, 2005). This is linked to the misconception that partial fragmentation involves separation and living area/habitat loss (Fahrig, 2003). Several studies about habitat fragmentation to date focussed on the ecological outcomes faced by organisms living in patches/land fragments emerging linked to the change in many land use forms (MacArthur and Wilson, 1967; Collingham and Huntley, 2013).

The fragmentation of forest ecosystems is often caused by inadequate forest management techniques and some socioeconomic issues (Sharma and Roy, 2007; Riitters *et al.*, 2002). Fragmentation often occurs due to urbanization, agriculture, deforestation for timber, land clearing, or natural causes like topographical and/or climatic conditions (snow and ice), fire, insects (Coops *et al.*, 2006), or invasion of non-forest plant species (Forman and Collinge, 1995; Babbar *et al.*, 2020). This causes a reduction in the forest area and the quality of the habitat (Carranza *et al.*, 2015; Sahana *et al.*, 2015; Batar *et al.*, 2017). According to Wulder *et al.*, 2009, the fragmentation of forests may cause irreversible destruction of biotic life due to genetic bottlenecks and abiotic elements within these ecosystems in the long term (Fischer and Lindenmayer, 2007). So, the deforestation of forests due to fragmentation is accepted as one of the primary causes of reduced terrestrial biodiversity (Long *et al.*, 2010; Mengist *et al.*, 2022).

Studying forest habitat fragmentation with a comprehensive approach – including its structure, function, and evolution – is a crucial part of the forest landscape management approaches and applications (Kerr and Ostrovsky, 2003; Linke *et al.*, 2006; Rose *et al.*, 2014). It represents the most visible dynamic aspect of forest stand evolution (Long *et al.*, 2010), that is a basic element of forest landscape change patterns at stand level in space and time (Innes and Koch, 1998; Healey *et al.*, 2018) and that can be modeled using integrated techniques of remote sensing and geographic information systems at different scales, from local to regional (Newton *et al.*, 2009; Healey *et al.*, 2018).

Forest fragmentation can be studied using a combination of comprehensive forest change analyses and time series of forest cover maps (Mengist *et al.*, 2022). In order to carry out fragmentation and corridor analysis, raw data such as satellite images or aerial photographs (Harper *et al.*, 2007) to be taken from two or more different time series belonging to the study area, as well as processed data such as stand maps, habitat maps, land use maps can be used as a baseline (Gergel, 2006). However, geo-statistical techniques and software tools enabled the generation of large volumes of new data from satellite imagery archives (Olariu *et al.*, 2022). The structural pattern of a landscape comprises three elements of matrix, patch, and corridor. Matrix is bro-ad areas formed by similar ecosystems or vegetation types. Corridors are defined as narrow lands. Patches are land surfaces that are non-linear and have different appearances than surrounding land (Weiers *et al.*, 2004). Patches may display differences in terms of location, size, shape, type, and boundary features; and their structures are very important in terms of the continuity of function and quality offered to eco-

systems (Theobald *et al.*, 2011). Forest clusters linked by corridors have preserved genetic diversity, and in this way, the survival capacity and sustainability of species increases. Providing connections between forest clusters, or strengthening existing connections has shown to be effective in ecology and land use management (Areendran *et al.*, 2020). The size and shape of forest fragments, the interior area contained within their boundaries, and available corridor systems are assessment criteria used for the status of forest habitats (Richard, 2011).

Biological corridors, which are developed as an alternative solution to the fragmentation problem, strengthen the interaction between forest fragments (Forman and Godron, 1986; Sharma and Roy, 2007). Biological corridors are forest clusters that ensure the integrity between forest fragments, and they have a significant potential to contribute to ecological and economic sustainability in forest ecosystems (Myroniuk *et al.*, 2020). Studying the fragmentation patterns will provide the foundation knowledge for management strategies. When creating or strengthening corridors, afforestation processes should begin after considering the topography, hydrological and soil characteristics, and the current threats to the area. It is important to thoroughly consider these factors to better support the policy and decision-making processes in forest management (Myroniuk *et al.*, 2020; Mengist *et al.*, 2022).

In this study, we attempt to guide forest managers by highlighting that fragmentation and corridor analyses can be used as tools in the management of forest fragmentation problems. Within the scope of this research, the fragmentation status, causes, and priorities of forest habitats in the study area were identified, and solution proposals were developed about creating integrated forest textures with the corridor/ connectivity approach.

2 Materials and methods

2.1 Study area

The study area in this research comprised Çankırı State Forest Enterprise (ÇSFE) located from 40° 30' and 41° 00' north latitude and 32° 30' and 34° 00' east longitude in north-central Turkey (Figure 1). A continental inland climate is found throughout the study area. Winters are harsh and cold and summers are hot and dry. Precipitation happens as snow in winter, with an annual precipitation sum of between 397-410 millimeters. The temperature range between +42 °C and -25 °C. In the study area, there are five types of large soil groups: alluvial, colluvial, chestnut-colored, brown forest, and non-calcareous brown forest soils. The soils in the region composed of many bare mountains and plateaus are under the threat of severe erosion. In consequence the study area cannot be cultivated as pasture. The habitat fragmentation status of the forest habitats within the boundaries of this study area was determined to test the connectivity between fragmented forest clusters using the corridor approach.



Figure 1: Location map of the study area.

Abbildung 1: Lage des Untersuchungsgebiets.

The study area, with a total size of 458,877 hectares, generally contains forests covering 76,206.9 hectares. The dominant vegetation of the study area is composed of forest trees such as larch, yellow pine, juniper, spruce, and fir, and fruit-bearing trees such as pear and dogwood. Conifer species occur in typically zoned belts with *Pinus nigra* found between 1100-1400 m, Pinus sylvestris between 1300-1700 m and *Abies nordmanniana* subsp. *equi-trojani* is located between 1500-1900 m above sea level. 43,256.8 hectares are productive forests and 32,950.1 hectares are forests with degraded quality. The forests experienced habitat degradation at varying degree in recent years linked to demands for different land uses by humans including road expansion, establishing agricultural areas, creation of settlement areas, development of mining sites, and dam construction. This situation carries a risk of disrupting the long-term sustainable use of forest assets (Sharma and Roy, 2007). It can cause quantitative and

qualitative disruption of habitat quality to be experienced within wildlife management (Srivastava and Tyagi, 2016). For example, in a wildlife survey covering the years 2016-2017 specific to the study area done by Çankırı Branch Office of Nature Conservation and National Parks, 16 large mammal species were found to live in this area. It was also observed that 12 of them were directly exposed to death or injury cases as a result of traffic accidents during 2015-19 years on highways, which is an indicator of habitat fragmentation in the region (Güven, 2019).

2.2 Data

In the first stage, the basic topographic maps including information like slope, aspect, and elevation of the study area were prepared with the ArcGIS 10.5 program. Then, the Coordination of Information on the Environment (CORINE) land cover data was used with the aim of identifying the temporal variation in forest assets and other land use in the study area by comparing data from 1990, 2000, 2006, and 2018. According to CORINE and European Environment Agency (EEA), land cover/use classification, and land cover/use data were produced with the visual interpretation method supported by a computer on the satellite images. According to EEA criteria and classification units (44 classes), variations in land cover/use observed on satellite images were identified with the aid of remote sensing and geographical information systems (GIS).

With the aim of investigating the current forest assets and revealing other habitat types in the study area, the current stand map for 2018 belonging to ÇSFE was used to prepare a European Union Nature Information System (EUNIS) habitat map. EUNIS defines habitat types in Europe and is a system to classify these habitats (EEA, 2021). The classification area is very large and is a habitat classification type accepted at a regional scale encompassing all land units and seas in Europe.

2.3 Methods

First of all, in order to determine the approximately 30-year areal change in both forest cover and all other habitat types, the areal data from CORINE and EUNIS for the years 1990 and 2018 were examined in terms of percentage change (Eq. 1).

Change between 1990 and 2018 = (Area 2018 - Area 1990)/ Area 1990

Then, for specific fragmentation analyses and corridor creation tests specific to forest habitats, structural assessment, network, and component-connection analyses were applied using the Graphical User Interface for the Description of image Objects and their Shapes (GUIDOS-Version 3.0) software program based on the ArcGIS program.

This program was developed by the European Commission Joint Research Centre, Institute for Environment and Sustainability.

GuidosToolbox actually contains a wide variety of generic raster image processing routines which include free software. These tools are based on geometric principles and can thus be applied at any scale and to any kind of raster data (Soille and Vogt, 2009; Vogt and Riitters, 2017). With the aid of this program, fragmentation analysis was performed for the study area in the first stage. The foreground area density (FAD) was analyzed on 5 separate observation scales in 6 classes for forest fragmentation in the GUIDOS software. These fragmentation classes were intact, interior, dominant, transitional, patchy, and rare. The rare class includes rare patches between fragmented forests, while the patch class involves scattered forest patches without clustering. The transitional class represents forest patches undergoing habitat change, while the dominant class involves the most frequently encountered forest patches in fragmented habitats. The interior class includes areas with a core of a certain size showing central features, while intact areas are forests with no change as a result of fragmentation (Gülçin, 2020).

GuidosToolbox includes morphological spatial pattern analysis (MSPA) which is a customized sequence for analyzing the connectivity of the image components. MSPA is based on mathematical morphology concepts and defines a single land cover map of the spatial relationships between land classes forming centers and connections (Soille and Vogt, 2009). To identify the center (core area) and connections (corridors) for MSPA, a range of image processing programs are used. Each forest pixel area in the input data only represents one geometric class (Vogt and Riitters, 2017).

In order to perform more advanced analyses using graphic-theory applications, MSPA analysis results are converted to network analyses. The components in network analyses comprise core and corridor areas. With this analysis, only a few components are present in the area. Area sizes for the core and corridors comprising these components were determined.

Then, component sensitivity analysis (connectivity importance) was used to calculate the ranked importance (sensitivity) of connectivity for each core and corridor area located in the study area. The connectivity importance was calculated with the related equations in the GUIDOS program (Saura and Rubio, 2010).

The map of the target area in the GUIDOS program was uploaded as 8-bit geotiff (raster). This map had 2 data classes foreground and background. Foreground data included forest areas, while background data included regions outside of the forest area. The forest polygons on these maps were converted to 30x30 resolution raster data with ArcMap and then assigned values of 2 bytes for the foreground data class and 1 byte for the background data class.

Program parameters comprised 4 basic variables.

- Variable 1 Foreground Data Connectivity: For foreground data (forest area), connections were assumed based on diagonal or lateral faces in 3x3 adjacent pixels. In this way, the areas between two fragments were classified as corridors instead of areas with a high threat of destruction.
- Variable 2 Margin width: This is used to determine the widths of areas that are not core areas. The margin width is determined by the pixel size of the map and the selected pixel numbers. In the program, the margin width was chosen as 4 pixels; in other words, 20 m.
- Variable 3 Transition areas: Transition areas are regions where interior or exterior margins intersect with corridors and where interior corridors intersect with core areas. This variable along with margin and interior margins represents closed boundaries.
- *Variable 4 Intext:* This variable ensures the differentiation of interior and exterior shapes.

After producing spatial data, more specific fragmentation analyses and corridor formation tests were begun unambiguously for forest habitats. To begin with, current fragmentation definitions are only descriptive, and as a result, quantifying the degree or changes in fragmentation for a given image is impossible (Vogt and Ritters, 2017). To quantify fragmentation, the foreground area density (FAD) approach was used from the fragmentation analyses in GUIDOS. FAD is calculated by measuring foreground density (P2) over five observation scales using a moving window analysis and foreground masking with square neighborhood areas of length 7, 13, 27, 81, and 243 pixels (Riitters et al., 2002, 2012a, 2012b).

Following the fragmentation analyses, Corridor-Connectivity Analyses of MSPA have been performed: MSPA statistics window displays basic statistics for seven fundamental MSPA foreground, missing, background, opening classes, etc. The left column of the table displays the percentage that equals the number of class pixels per foreground area and per data area. The frequency equals the number of unique objects of the given class and, where applicable, the area equals the number of background pixels covered as shown in the right column.

Table 1: Area by land cover types from CORINE land cover from years 1990, 2000, 2006 and 2018 and the percent changes in area between 1990 and 2018.

Tabelle 1: Fläche der Bodenbedeckung aus CORINE Landbedeckung für die Jahre 1990, 2000, 2006 und 2018 sowie die prozentuelle Änderung der Fläche zwischen 1990 und 2018.

Code	Description	Area 1990 (ha)	Area 2000 (ha)	Area 2006 (ha)	Area 2018 (ha)	Change in % between 1990-2018
111	Continuous urban fabric	43	43	117	117	+ 171
112	Discontinuous urban fabric	2750	2750	2752	3044	+ 11
121	Industrial or commercial units	305	451	425	750	+ 146
122	Road and rail networks and associated land	70	0	0	30	-57
133	Construction sites	0	0	51	26	+ 26
141	Green urban areas	0	0	34	34	+ 34
211	Non-irrigated arable land	107691	108471	103659	110799	+ 3
212	Permanently irrigated land	31996	31970	32094	26865	-16
213	Rice fields	6760	6810	6799	7138	+ 6
221	Vineyards	280	280	0	0	-100
222	Fruit trees and berry plantations	48	48	186	186	+ 287
231	Pastures	7542	6585	7538	5365	-29
242	Complex cultivation patterns	22053	22080	19423	18988	-14
243	Land principally occupied by agriculture with significant areas of natural vegetation	61942	61740	57948	55645	-10
311	Broad-leaved forest	1070	738	489	518	-52
312	Coniferous forest	18952	19898	21988	22277	+ 18
313	Mixed forest	1655	2003	949	838	-49
321	Natural grasslands	92136	92364	101958	104446	+ 13
324	Transitional woodland-shrub	19913	18950	21145	27529	+ 38
331	Beaches dunes sands	1815	1803	1527	1400	-23
332	Bare rocks	20983	20983	19019	9226	-56
333	Sparsely vegetated areas	61902	61902	62084	64474	+ 4
411	Inland marshes	1111	1111	841	840	-24
511	Water courses	992	977	963	853	-14
512	Water bodies	216	268	236	839	+ 289
	TOTAL	462225	462225	462225	462225	0

3 Results

With the aim of testing temporal change of the forestry assets and other land use features in the study area, CORINE satellite images were used with distribution maps prepared for land cover classes for the years 1990, 2000, 2006, and the most up-to-date data from 2018. Then the variations in land cover classes were tested for the periods 1990-2000, 2000-2006, and 2006-2018 with the aim of periodically observing changes to the land use structure during this time (Table 1).

Finally, all these changes observed in land cover from 1990-2018 were summarized and investigated in depth specific to the existence of forests (Figure 2, Table 2).

Table 2: Comparison of 1990-2018 forest area based on CORINE land cover.

Tabelle 2. Vergleich der Waldflächen	1000-2018 auf der Grundlage der CORINE Landbedeckung
Tabelle 2. Vergielch der Waldhachen	1990-2016 auf der Grundlage der COnine Landbedeckung.

Code	Area 1990 (ha)	Area 2018 (ha)	Change in % between 1990 and 2018
311 (Broadleaf forests)	1070	518	-52
312 (Coniferous forests)	18952	22277	+ 18
313 (Mixed forests)	1655	838	-49
324 (Transitional woodland/shrub)	19913	27529	+ 38
TOTAL	41590	51162	+ 23





Figure 2: Changes of forest area in the study area based on CORINE land cover for 1990 (a) and 2018 (b).

Figure 2: Veränderung der Waldfläche im Untersuchungsgebiet unter Verwendung von CORINE Landbedeckungskarten für 1990 (a) und 2018 (b).

As CORINE data only contains 44 classes for classification units using a variety of criteria based on pure remote sensing, relatively rough data could be obtained about habitat structures (Ürker and Özen, 2020). For this reason, the current stand maps (ÇSFE, 2021) for 2018 produced during forest management planning based on data measured in the field were used as a digital substrate, and habitat characteristics were assessed in more detail under the EUNIS classification system (EEA, 2021). EU-NIS habitat types were extracted at the second level based on the 2004 revision of the EUNIS habitat classification (Table 3, Figure 3).

To separately assess the current status of forest texture among all habitat types, spatial data, and habitat patch numbers were compared with each other for all habitat types in the study area (Table 3).

Table 3: 2018 EUNIS Habitat Types.

Tabelle 3: 2018 EUNIS-Lebensraumtypen.

		Number of habitat
EUNIS Codes of Habitat Types	Area (ha)	patches
C1 - Surface standing waters	684	64
C2 - Surface running waters	688	31
E1.2E - Irano-Anatolian steppes	112642	868
E2.6 - Agriculturally-improved, re-seeded and heavily fertilized grassland, including sports fields and grass lawns	70681	289
F9.31 - [Nerium oleander], [Vitex agnus-castus] and [Tamarix] galleries	4520	336
G1.37 - Irano-Anatolian mixed riverine forests	1455	42
G1.7A2 - Irano-Anatolian steppe [Quercus] woods	25615	341
G1.A1 - [Quercus] - [Fraxinus] - [Carpinus betulus] woodland on eutrophic and mesotrophic soils	6	1
G1.A72 - Sub-Euxinian mixed [Quercus] - [Carpinus betulus] forests	132	2
G1.D2 - [Juglans] groves	7	1
G3.17 - Balkano-Pontic [Abies] forests	2369	30
G3.4E - Ponto-Caucasian [Pinus sylvestris] forests	5337	159
G3.4F - European [Pinus sylvestris] reforestation	193	3
G3.5 - [Pinus nigra] woodland	32813	408
G3.57 - [Pinus nigra] reforestation	3235	99
G3.75 - [Pinus brutia] forests	83	6
G3.9C - [Cedrus] woodland	1074	79
G4.4 - Mixed [Pinus sylvestris] - [Betula] woodland	242	27
G4.7 - Mixed [Pinus sylvestris] - acidophilous [Quercus] woodland	128	1
G4.71 - Subcontinental nemoral [Pinus] - [Quercus] forests	9698	277
G4.8 - Mixed non-riverine deciduous and coniferous woodland	630	27
H5.37 - Boulder fields	246	5
H5.52 - Sparsely vegetated burnt areas	305	7
I1.1 - Intensive unmixed crops	179903	1269
J1.2 - Residential buildings of villages and urban peripheries	8996	319
J2.2 - Rural public buildings	7	2
J3.2 - Active opencast mineral extraction sites, including quarries	322	40
J4 - Transport networks and other constructed hard-surfaced areas	3	1
J4.7 - Constructed parts of cemeteries	210	59
TOTAL	462225	4793



Figure 3: EUNIS habitat map for the study area in 2018.

Figure 3: EUNIS-Lebensraumkarte für das Untersuchungsgebiet im Jahr 2018.

According to FAD analyses, there were no intact forest patches; forest areas with a core area of a certain size comprised 15.5%, which was very consistent with MSPA values; habitat transition was observed in 15.88% of the area; and scattered forest patches covered 3.81% of the total area (Table 4, Figure 4).

Before beginning MSPA analysis, simplified pattern analysis (SPA) was performed. Of the 458,877-hectares study area, 69,562 hectares comprised core areas (15.5%), 9268 hectares were exterior margins (2%), 1180 hectares were areas with high destruction threat and 218 hectares (0.05%) were corridors (Table 5).

According to upper spatial class analysis comprising component, core area, and corridor classes, the study area was identified to contain 1511 forest components (Table 6). The degree of significance was created for a total of 1511 components according to corridor and core areas.

According to Table 6, 0.10% of the foreground area is made up of islet pixels, while 0.02% of the area (= Foreground + Background) is made up of islet pixels, there are also 107 islets (regardless of their own size).

Similarly, all statistics for class perforation reveal that the image contains 314 perforations in total, and entire perforation pixels together makeup 2.17% of the foreground area or 0.39% of the data area (foreground area + background area).

Table 4: Fragmentation classes for forest in the study area.

Tabelle 4: Fragmentierungsklassen für den Wald im Untersuchungsgebiet.

Foreground area						
density (FAD):	1					
FragmClass\ObsScale:	(7pix)	2 (13pix)	3 (27pix)	4 (81pix)	5 (243pix)	Summary
	0.0021					
Rare: FAD <10%	68	0.012466	0.127374	0.848583	2.39182	0.001301
	1.4817					
Patchy: 10≤FAD<40%	7	2.99703	6.02073	14.6635	30.5022	3.81851
Transitional:	6.5925					
40≤FAD<60%	6	9.54472	14.5733	23.333	31.4493	15.8862
Dominant:	19.346					
60≤FAD<90%	1	28.8573	38.4128	48.3906	34.7041	64.7892
Interior:	11.857					
90≤FAD<100%	7	19.8802	25.5826	11.859	0.95265	15.5047
	60.719					
Intact: FAD=100%	6	38.7082	15.2832	0.905278	0	0



Figure 4: Fragmentation map (above) for forest in the study area and summary graph (below) of fragmentation classes.

Figure 4: Fragmentierungskarte (oben) für den Wald im Untersuchungsgebiet und zusammenfassende Grafik (unten) der Fragmentierungsklassen.

Table 5: Table of Simplified Pattern Analysis (SPA) for the study area.

labelle 5: labelle der vereinfachten Musteranalyse (SPA) für das Untersuchungsgebiet.

Total area	Core area		External edg	ge	Corridor pres	ent	Areas with hi destruction	gh threat of
ha	%	ha	%	ha	%	ha	%	ha
458877	15.1591821	69562	2.01971334	9268	0.04750728	218	0.25714952	1180

Table 6: Summary of Morphological Spatial Pattern Analysis (MSPA).

Tabelle 6: Zusammenfassung der morphologischen Raummusteranalyse (MSPA).

	Foreground (FG) / data [%]	# / Background (BG) area
CORE(s)	/	0
CORE(m)	83.68/15.14	910
CORE(1)	/	0
ISLET	0.10/ 0.02	107
PERFOR	2.17/ 0.39	314
EDGE	11.71/ 2.12	628
LOOP	0.19/0.03	415
BRIDGE	0.27/ 0.05	500
BRANCH	1.88/ 0.34	6403
Background	/81.91	1511/417620
Missing	39.31	2/3304238
Opening	94.14 Integrity	1413/57384
CoreOpen	/0.69	639/35388
BorderOpen	/ 0.43	774/21996

4 Discussion

4.1 Assessments of the analyses of fragmentation and connectivity on forest habitats

According to Table-3 and Figure-3, 6 different main habitat types including 44 subhabitat types were determined. When the general CORINE data are investigated, areas belonging to the urban ecosystem like continuous and temporary settlement areas, and industrial-commercial units increased in stages from 1990 to the present. Though spatial variation in agricultural areas displayed variations through the years, in a cumulative sense no serious change was notable (Bascompte *et al.*, 2002). When shores-beaches-dunes (on the shores of rivers), swamps, water routes, and water masses are assessed, though a yellow undulating variation was observed through the years, no serious spatial variation trend was experienced specific to aqueous habitats linked to rivers in the region when assessed cumulatively. Though pasture areas are reduced, when natural meadows, coniferous forests, broad-leaf forests, transitional woodland areas, and mixed woodland are considered, an increase in all forestry assets is notable. However, specific to sub-units, a low rate of broad-leaf woodland was converted to agricultural areas.

To compare the forest assets and use in the past with the present day, to investigate the impact of anthropogenic constraints on habitat fragmentation, and to measure the impact and effectiveness of forest villages on habitat fragmentation, CORINE satellite images from 1990-2018 were used for detailed examination of the changes in habitat types/land use structure. When the data are evaluated specific to forest habitats, firstly, the 41590 hectares forest area in 1990 was identified to reach 51162 hectares with a 23% spatial increase cumulatively up to 2018. When the CORINE units are assessed separately, nearly half of the broad-leaf woodland and mixed woodland were destroyed, while transitional woodland increased by 17.5% and coniferous woodland increased by 38.2%.

The most important causes of the stepped increase in forest assets over time in the study area are agricultural land abandoned due to external migration converting to forestry, and forest margins and forest openings becoming forestry as a result of afforestation-maintenance-rehabilitation work. The most basic cause for the relative increase in coniferous woodland assets is that coniferous species have been dominantly used in regular afforestation-maintenance-rehabilitation work completed over the last 30 years.

When the CORINE data (extracting codes related to forestry) from 1990 to 2018 were compared, the general forestry assets in the ÇSFE appear to have a clear net increase of 23%. According to Table 2, during nearly 30 years, the presence of broad-leaved and mixed forests was both reduced by nearly 50%, while the presence of transitional woodland/shrub increased by 38% and coniferous forests increased by 18%.

Contrary to the 23% net increase in woodland spatial area in the last 30 years, it is interesting that nearly all forest textures were identified to have habitat fragmentation (Biswas and Khan, 2013). One of the main reasons for this is mistaken afforestation work not paying attention to landscape ecology principles (Fahrig, 2003; Collingham and Huntley, 2013).

Considering Table 3, the general status is that 83017 hectares of the 462225-hectares study area – in other words, nearly 20% - is covered with forest habitats with different characteristics. When data are examined in terms of the number of habitat patches, forest textures existed in 1839 of 4793 habitat patches and this encompasses nearly 40% of this area.

When both Table 4 and Figure 4 are investigated, margin effects were present at a very high rate of 64.78% of the fragmented forest texture in the study area.

The overall area of the background contained by perforation pixels (core openings) is 35388 pixels. All MSPA statistics were computed for foreground borders that were closed. Forest integrity is a statistic that takes into consideration the total area of all openings in a forest (= forest + openings). If there are no openings in the forest, its integrity is 100%. According to Table 6, 5.86% of the integrated forest area has openings, implying that forest integrity is reduced to 94.14%.

The foreground region has 1413 apertures with a total count of 57,384 pixels. 639 of the 1413 openings are core openings within the forest's core region, representing a total area of 35,388 pixels (inside the perforations). Border vacancies account for the remaining 774 openings had a total size of 21996 pixels at the outside forest boundary (edge). The following network options were available after MSPA analysis. In Saura and Rubio (2010), the connection significance is computed using Equation 4. Accordingly, the corridor connections, interaction areas, connection zones, and network components specific to the study area are summarized on the map below (Figure 5). According to this network analysis, there are 275 individual components in the network. The equivalent connected (node/core) area (ECA; measured in area units, such as hectares, or in the general instance, pixels) is 229,173 pixels. The degree of network connectivity (DOC) is 29.69%.



Figure 5: General corridor map of the study area according to MSPA analysis.

Figure 5: Allgemeine Korridorkarte des Untersuchungsgebiets gemäß der MSPA-Analyse.

As a result of the disrupted fragmentation in the study area in general and the increase in distance between forest patches, the ecological integrity, and communication between forest patches are lost (Backhaus *et al.*, 2002). As a result, both plant species and wildlife are trapped in distant forest patches (Forman and Collinge, 1995). In a general sense, this is one of the reasons why biodiversity is endangered in general and is a situation that planners should consider (Bürgi, 1999; Sharma and Roy, 2007). It is necessary to connect separated areas with corridors to ensure the mutual transition of species (Batar *et al.*, 2017). The presence of many patches is due to afforestation activities in these areas. For this reason, forest management chiefs linked to Çankırı Forestry Management Directorate should ensure patches are joined during renewal processes in forestry management plans, and as a result, forest integrity should be ensured (Srivastava and Tyagi, 2016). Additionally, with the aim of ensuring linkages and continuity of the patchy forest ecosystem, the forest ecosystem should be improved by uniting patchy areas (Saura and Rubio, 2010; Theobald *et al.*, 2011; Srivastava and Tyagi, 2016; Hu *et al.*, 2020).

4.2 Contributions of the survey studies

During the field controls in the area, a 24-item survey was administered to headmen in 25 different forest villages and the findings were assessed with descriptive analyses in the SPSS program. When the headmen participating in the survey were requested to assess the temporal variation in forestry for their region, 32% stated that no change had been experienced while 52% stated that there was an increase in forestry, contrary to a reduction. They indicated that the underlying causes for this were the slow transformation to the woodland of agricultural areas following reduced human intervention after being abandoned and the afforestation-maintenance work performed in forest openings and margins by forest management units.

When participants were questioned about the relative change compared to past years in the use of relationships for forests in their area, 92% of headmen participating in the survey stated that these relationships had reduced a lot. When questioned about the reasons underlying this change, the most important cause was stated to be external migration during the last 30-40 years (92%). Among these responses, it is notable that no participant marked choices related to restrictions on the use of the forest by the private sector (mining, energy, dam, etc. projects). When answers to another question about the variation of wildlife in forestry in the area are examined, though there was a choice for wildlife has much reduced, no headman marked this, but most stated that wildlife had visibly increased (96%). When responding to this guestion, headmen stated that wild boars had increased a lot, followed by roe deer observed with the increase in forestry in recent years, while herbivore and carnivore mammals began to be seen intensely linked to the construction of artificial ponds in the region. When guestioned about whether judicial-administrative cases involving punitive procedures were experienced related to the forestry in the areas, 88% of headmen participating in the survey stated that none of these cases were currently known, while 12% stated that very few isolated cases were known at present. Most of these cases occurred linked to illegal tree cutting or illegal grazing.

5 Conclusions

In this study, the aim was to determine the fragmentation status of forest habitats within ÇSFE boundaries located in the north-central region of Turkey and to create infrastructure to strengthen the connectivity between fragmented forest clusters with the corridor approach (Batar *et al.*, 2017).

With the integration of analysis results obtained as a result of the research with the forestry management plans of ÇSFE, the potential to benefit from this data when updating these plans is high (Saura and Rubio, 2010). In this situation, in addition to direct short-term economic contributions to the forestry area, improvements will be provided for habitat qualities of living organisms linked to the forest and wildlife elements in the region in the long term and it will be possible to ensure maximum benefit in terms of ecosystem services (Collingham and Huntley, 2013; Batar *et al.*, 2017).

Areas between forest patches located in the study area comprise open areas for agriculture, forest soil (FS), and pastures. From this aspect, these patches should be joined over time with afforestation studies to create ecological corridors and thus, some areas will be allocated as ecological interaction zones (Srivastava and Tyagi, 2016). For this reason, it is necessary to give priority to afforestation studies for this type of area.

If the afforestation studies that are recommended to be updated in the light of the corridor approach developed by taking into account the fragmentation and connectivity analyses obtained from this study, are implemented, it will be possible to ensure long-term sustainable management in light of landscape ecology principles about forest structures with different fragmentation conditions in different regions (Forman and Collinge, 1995; Fahrig, 2003; Hanski, 2005; Yadav *et al.*, 2020) not only in the study area but in Turkey and the surrounding geography.

Author's Contribution

All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by Okan Urker, Alkan Gunlu, and Murat Ataol. The first draft of the manuscript was written by Okan Urker and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript. Research and publication ethics were complied with in the study. We declare that the figure or figures obtained from external sources within the study are materials that do not require copyright permission, by citing the relevant source. All authors read and approved the final manuscript.

Competing Interests

The authors declare that they have no competing interests or conflicts of interest.

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