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Spatial distribution and association patterns of *Pinus brutia* Ten. regeneration

Räumliche Verteilung und Assoziationsmuster von *Pinus brutia* Ten. Verjüngung

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Keywords: *O-ring statistics, parent trees, natural regeneration, understory*

Schlüsselbegriffe: *O-Ring Statistik, Elternbäume, Naturverjüngung, Unterschicht*

Abstract

Pinus brutia Ten. forms a distinctive natural coniferous forest in Kurdistan region in Iraq. The aim of this study was identifying the spatial distribution and association patterns of *P. brutia* regeneration. On September 2016, 10 study plots with a total area of 0.05 ha of each plot were set up along a 2 km transect in the northern aspect of Zawita natural forest and the positions of *P. brutia* parent trees, regeneration and shrubs were recorded. We used O-ring statistics to recognize the spatial distribution and association patterns of *P. brutia*. Spatially, seedlings and saplings of *P. brutia* were distributed in an aggregation pattern in comparison to the randomized pattern of the parent trees. A pattern of repulsion was found between seedlings and saplings with parent trees. The number of seedlings decreased with the increasing stand basal area, but in a positive correlation with the presence of shrubs and with the increasing shrub crown area, particularly with *Quercus* shrubs. Both patterns of early regeneration of *P. brutia*, spatial aggregation as well as repulsion from parent trees mainly derived from the competition with parent trees.

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Zusammenfassung

Pinus brutia Ten. bildet einen ausgeprägten natürlichen Nadelwald in der Kurdistan-Region im Irak. Das Ziel dieser Studie war die Ermittlung der räumlichen Verteilung und der Assoziationsmuster von *P. Brutia* Verjüngung. Im September 2016 wurden 10 Probeflächen mit einer Fläche von je 0.05 ha entlang eines 2 km langen Transsekts im nord-exponierten Teil des Zawita-Waldes festgelegt und die Positionen von *P. brutia*-Elternbäumen, der aufkommenden Verjüngung und von Sträucher aufgezeichnet. Die räumlichen Verteilung und Assoziationsmuster von *P. brutia* wurden anhand von O-Ring-Statistiken untersucht. Während die Positionen der Elternbäume eher zufällig sind, zeigten die Keimlinge und Jungpflanzen ein deutliches Aggregationsmuster. Wir konnten eine Abstoßungsreaktion zwischen Keimlingen und Jungpflanzen und den Elternbäumen feststellen. Die Anzahl der Keimlinge sank zwar mit steigender Baumgrundfläche, stand aber in positiver Korrelation mit dem Vorhandensein und der Kronenfläche von Sträuchern, insbesondere mit *Quercus*-Sträuchern. Diese Muster der frühen Verjüngung von *P. brutia* – die räumliche Aggregation sowie die Abstoßung (Repulsion) von Elternbäumen – entstehen hauptsächlich durch die Konkurrenz mit Elternbäumen.

1. Introduction

One main aim of forest ecosystem studies is to understand the processes and mechanisms that drive the regeneration of tree species (Jia et al., 2016). Patterns in the distribution of regeneration are essential for establishment of regeneration. The spatial pattern of tree regeneration has been increasingly recognized (e.g., Muhamed et al., 2015; Ghalandarayeshi et al., 2017) and investigation of the effect of spatial interactions in population and community dynamics is a focus of current forestry research (McIntire & Fajardo, 2009). Advanced ecology is driven by an interest in processes that are inherently spatial, as in the case of plants. Under the assumption that processes generate patterns, intra- and inter-specific interactions between species can produce spatially recognizable patterns (Rayburn & Monaco, 2011; Kang et al., 2017). Spatial distribution patterns become apparent in the aggregation of plants. That means that plants can be aggregated (clustered) or positioned in the opposite formation, which is a systematic (regular) order. If neither of those applies, they are in random arrangement (Clark and Evans, 1954). The aggregation only relates to the spatial location, regardless of the involved species. When considering species the terms association versus segregation of plants can be used. Association means that it is more likely that the nearest neighbor of a plant is an individual of a different species than of the same species. In contrast, segregation means that different species grow in homogeneous groups (Pielou 1961).

The main aggregation patterns observed at small spatial scales have been referred to as either positive (facilitation) or negative (competition) plant-plant interactions. For example, nearly regular plant spatial patterns are usually a sign of intense competition between plants for limited resources like water and light (Stoll & Bergius, 2005; Rayburn & Monaco, 2011). While, aggregated patterns (especially interspecific) may be an indication of positive plant interactions at small scales, whereby the presence of one species alleviates a severe abiotic environment for other species (Gómez -Aparicio et al., 2005; Kefi et al., 2007). If the processes that cause segregation or aggregation are absent, or present but in balance, then random patterns are generated (Szwagrzyk & Czerwczak, 1993).The aggregated distribution of species is a common pattern, particularly for naturally regenerated seedlings in forests (Zhang et al., 2013). The location of parent trees and the modes of seed dispersal usually determine the spatial patterns of seed rain (Hubbell, 2001). Seedling establishment is then influenced by successive post-dispersal biotic and abiotic factors (Gholami et al., 2018). Niche segregation (Pielou, 1961), habitat heterogeneity (Gholami et al., 2018) and plant-plant interactions are considered to be the mechanisms that contribute to aggregation patterns of regeneration. The patterns resulting from spatial distributions and associations may vary among species, e.g., species-specific, and over a time gradient, e.g., the developmental stage, and when species grow in heterogeneous habitats (Zhang et al., 2013; Muhamed et al., 2015; Wiegand et al., 2017).

In water-limited ecosystems typical found under dry continental Mediterranean growing conditions, natural forest regeneration is severely limited at all stages, with high mortality rates among both natural and planted seedlings (Gómez -Aparicio et al., 2005; Padilla & Pugnaire, 2006). Under such environmental stress, parent trees and shrubs have been recognized as important niches for tree regeneration (Callaway, 1997; Rodríguez-García et al., 2011b). Among the facilitative effect of the presence of an overstory canopy for seedlings, the most positive effects are interspecific while the negative effects are intraspecific e.g., competition between the need for water or nutrients and light by parent trees versus offspring regeneration. Studies in Mediterranean ecosystems have investigated the influences of canopy cover on the natural regeneration mechanisms of many pine species like *Pinus sylvestris* L. (Barbeito et al., 2009), *Pinus pinaster* Ait. (Rodríguez-García et al., 2011a), *Pinus halepensis* Mill. (Prévosto et al., 2012), *Pinus pinea* L. (Adili et al., 2013), and more recently *Pinus nigra* (Calama et al., 2017) and *Pinus brutia* Ten. (Muhamed et al., 2018). The aforementioned studies indicated increasing seedling mortality in summer and in open areas and provide evidence for positive correlation with shrubs, while parent tree – regeneration association patterns varied between positive under partial canopy cover and moderate shade to clear negative association patterns. In the Kurdistan region, Zawita pine (*Pinus brutia* Ten.) is distributed in small enclaves and its many physiological and morphological features mean it is well-adapted to the Mediterranean-type climate (Thanos & Scordilis, 1987). In Iraq, it is one of the most widely used species for planting in dry and semi-dry lands due to its limited ecological requirements and high tolerance to drought and heat. The light requirements of its seedlings are high. According to Özdemir (1977) this species regenerates poorly under low light intensity, i.e. below 65–70 %. It occupies open disturbed sites, generally with a sparse vegetation cover, and particularly in the absence of aggressive competitors. Therefore, the heliophilous nature of the species exploits open habitats produced by fire through massive recolonization (Thanos & Doussi, 2000; Boydak, 2004; Muhamed et al., 2018). Detecting forest spatial patterns at each of the development phases may provide information on changes in plant-plant interactions from one development stage to the other, thereby uncovering the processes underlying forest future regeneration dynamics (Kazempour et al., 2018). One way to explore the spatial dynamics of forest regeneration without long-term demographic censuses is to perform a point pattern analysis based on analysing fully mapped tree positions using, for example, the centroid positions of trees or shrub stems or shrub crowns in relation to other forest understory species (Getzin & Wiegand, 2007; Muhamed et al., 2015) In forestry, the impetus for using point pattern analysis to detect tree spatial patterns is reasonable because, compared to other plant communities like diffuse growing shrubs and grass, forests comprise a wide point pattern that in many cases can be approximated by dots (Wiegand & Moloney, 2004).

To our knowledge, little is known about the regeneration dynamics of *P. brutia*, in Iraq, in particular on the role of parent trees and shrubs in the regeneration process. In this study, we intend to answer the following questions: What are the prevailing types of spatial distribution and association patterns in three life stages of trees in Zawita forest? Is it possible to detect mechanisms that help shape the outcome patterns of this species? The findings from this study will help understand the ecological requirements of *P. brutia* regeneration over life stage gradient and support the theoretical basis of *P. brutia* regeneration and community succession.

2. Materials and methods

2.1. Study site

The study was conducted in the northern aspect of the Zawita–Duhok province, Kurdistan region-Iraq, (N 36° 53′ 48″ E 43° 08′ 48″) at elevations ranging from 900 m up to 1600 m.a.s.l. (Figure 1). Average total rainfall is 578.5 mm and the mean annual temperature is 15 °C. Monthly average relative humidity (RH %) ranges from 18.5 % to 68% with a total average of 44.18% (Directorate of Meteorology 2014). Soils are acidic (mean pH 5.0) and poor in nutrients, especially in N, P, K+, Na+, Ca2+, Mg2+, Mn2+, Fe2+, Zn2+, and Cu2+ compared to the dominant oak forest in the area. The study site contains a ridge that is 70 % covered by *Pinus brutia* with some associated species, particularly sclerophyllous ones like *Juniperus oxycedrus*, *Paliurus spina-christi*, *Quercus spp.* and *Pistacia eurycarpa*, *Pistacia khinjuk*, *Crataegus monogyna*, *Prunus microcarpa*, *Anagyris foetida*, *Rhus coriaria*. While a comprehensive fauna survey is not conducted yet in Zawita, there is evidence for a diverse population of mammals, reptiles and birds. Zawita is famous for its pine forests commonly found in fire-related ecosystems of the eastern Mediterranean region. Zawita is considered by many authors to be the southern limit of the distribution of this species in the eastern Mediterranean (Biger & Liphschitz, 1991). Today, the pine forests of Zawita are showing signs of deterioration, as, in addition to forest fires, human recreational activities and urbanization are major threats to *P. brutia* forests, which could mean that natural regeneration is not sufficient to replace mature and over-mature trees.

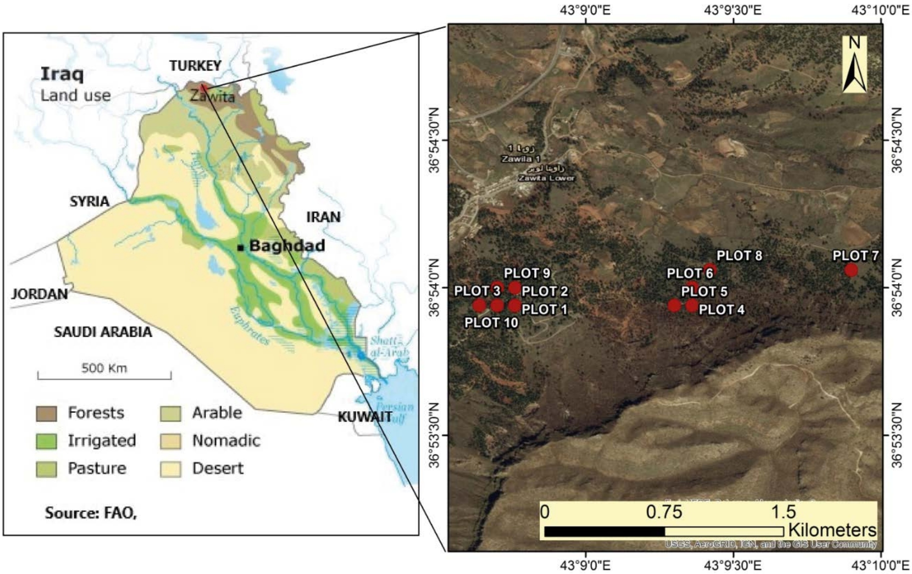

Figure 1: Location of the study site and plots in Zawita-Duhok province. Maps were generated using ArcGIS 10.0.

Abbildung 1: Lage des Untersuchungsgebiets und der Probeflächen in der Zawita-Duhok-Provinz. Karten wurden mit ArcGIS 10.0 erstellt.

2.2. Species

P. brutia is restricted to a small north-eastern corner of the Mediterranean region. It grows from Greece to Iraq, but is mainly concentrated in Turkey and Cyprus. Dar Kaja Zawita or Sanoubar Zawita is local common names for *P. brutia* in Iraq. Is a medium size tree, reaching 20–35 m in height and with a trunk diameter of up to 1 m. The species naturally cover about 10,000 hectares distributing in Zawita and Atrush-Duhok districts (Thanos & Scordilis, 1987). *P. brutia* cannot resprout and regenerates from seed. It is light-demanding, grows rapidly in its early establishment phases and is a site-insensitive species that can adapt to most soil types as well as being tolerant to drought (Urgenc, 1971; Quézel, 2000; Boydak, 2004). In Iraq, *P. brutia* has been widely planted as a landscape tree in urban areas and for the revegetation of bare watersheds. Silvicultural experience obtained from local afforestation practices suggests that to achieve reasonable growth in arid and semi-arid zones, up to three years of irrigation are recommended after planting, particularly during summer.

2.3. Sampling and data analysis

2.3.1. Sampling methods

In the middle of September 2016, 10 rectangular plots with size 25 m by 20 m were allocated along a 2 km transect. We assumed this plot size is sufficient to ensure homogeneity in site conditions within the plot and a sufficient number of regeneration, parent trees and shrubs. For the stem census and to facilitate data collection, each plot was further divided into twenty 5 by 5m quadrates. In each of the 200 quadrate plots, all living seedlings (h ≤ 50 cm), saplings (h > 50 cm and diameter at breast height DBH < 4 cm), trees (DBH > 4 cm), and shrubs (crown diameter > 30 cm) were mapped to reference axes with origins (x, y = 0, 0) located in the lower right corner of the plot using a measuring tape (±5 cm) in a Cartesian coordinate system (Muhamed et al., 2015) (Figures 2a, 2b and Figure A2 to A8 in Appendix). The long diameter width and short diameter length were used to quantify the crown area (CA) of each pine tree and shrub within an elliptical crown shape. The stand basal area BA in m² of each plot was calculated according to Eq. 1, where *d* represents the tree diameter at breast height.

$$\mathbf{BA = 0.00007854 * \boldsymbol{d^2}} \qquad \text{(Eq. 1)}$$

2.3.2. Data analysis

The univariate O-ring statistic was used to investigate the pure spatial distribution (aggregated, regular and random) regardless of the species, in two categories: parent trees among each other, regenerated trees (seedlings, saplings) among each other. The bivariate O-ring statistic was used to detect the association patterns of parent trees with seedlings and saplings. The shrub-seedlings/saplings were spatially also detected once with all shrub species combined and once with specified shrub species (Figure A1). As defined by Wiegand & Moloney (2004), the bivariate O-ring statistic O12 (r) (Eq. 2) gives the expected number of points in a pattern 1 (e.g., seedlings) at distance r from an arbitrary point of pattern 2 (e.g., trees) under the assumption of the antecedent condition null model (Wiegand & Moloney, 2004). This model randomizes the locations of the pine regenerations assuming that they could be anywhere in the plot and keeps the locations of the adult trees and shrubs fixed assuming, first, that their location did not change during pine regeneration establishment and second, that larger trees influence the distribution of the small pine regenerated individuals but not vice versa.

$$\mathbf{O12(r)} = \frac{\sum_{i=1}^{n1} \mathbf{Points2}\left[\mathbf{R}_{1,i}^{\mathbf{W}}(\mathbf{r})\right]}{\sum_{i=1}^{n1} \mathbf{Area}\left[\mathbf{R}_{1,i}^{\mathbf{W}}(\mathbf{r})\right]} \qquad \text{(Eq. 2)}$$

n1 is the number of points in pattern 1,

$$R_{1,i}^{W}(r)$$

is a ring with width W at distance r from an arbitrary point of pattern 1,

$$\sum_{i=1}^{n1} \text{Points2}\left[R_{1,i}^{W}(r)\right]$$

is the number of points in pattern 2 in

$$\left[R_{1,i}^{W}(r)\right] \text{ and } \sum_{i=1}^{n1} \text{Area}\left[R_{1,i}^{W}(r)\right]$$

is the area of

$$R_{i}^{W}(r).$$

$$R_{1,i}^{W}(r)$$

is the ring with radius r and W width centred at the ith point of pattern 1, n1 is the total number of points of pattern 1 in the study region

$$R_{1,i}^{W}(r).$$

Since we are detecting the distribution patterns of the same species cohort e.g, seedling, the univariate O-ring statistic O11 (r) is estimated by setting pattern 2 equal to pattern 1 under the assumption of complete spatial randomness (CSR) where the points are independently and randomly distributed over the entire plot (Wiegand & Moloney, 2004). In this study, we defined three fine scale spatial patterns. For the spatial distribution of parent trees and regeneration trees 10 m was selected, for the association of type shrub-regeneration 2 m was selected, for the association of type parent tree-regeneration 10 m was selected with 0.25 m intervals. All point pattern analyses were performed with the grid-based software Programita (Wiegand & Moloney, 2004, version updated Feb. 2014 from http://programita.org/., settings: ring width = 0.25, cell size = 1 m). A goodness-of-fit (GoF) test was used to avoid the risk of inflating type I errors that may occur when the value of O (r) is close to a simulation envelope (Grabarnik et al., 2011). Reducing the plant size to dot locations is one of the limitations of point pattern analysis, particularly when the plant is bigger than the spatial scales investigated. This may mask real spatial relationships at smaller scales that represent the scales that interest most ecologists who study plant-plant interaction (e.g., Wiegand et al., 2006; Muhamed et al., 2015). Therefore, to confirm our spatial patterns, a second-order polynomial models (Microsoft® Office Excel 2007) was performed to describe the effect of tree and shrub density parameters (stand basal area, the tree crown area, number of trees, shrub crown area and the number of shrubs) on total seedling and sapling density. One way ANOVA was used to detect the significance of the correlation patterns.

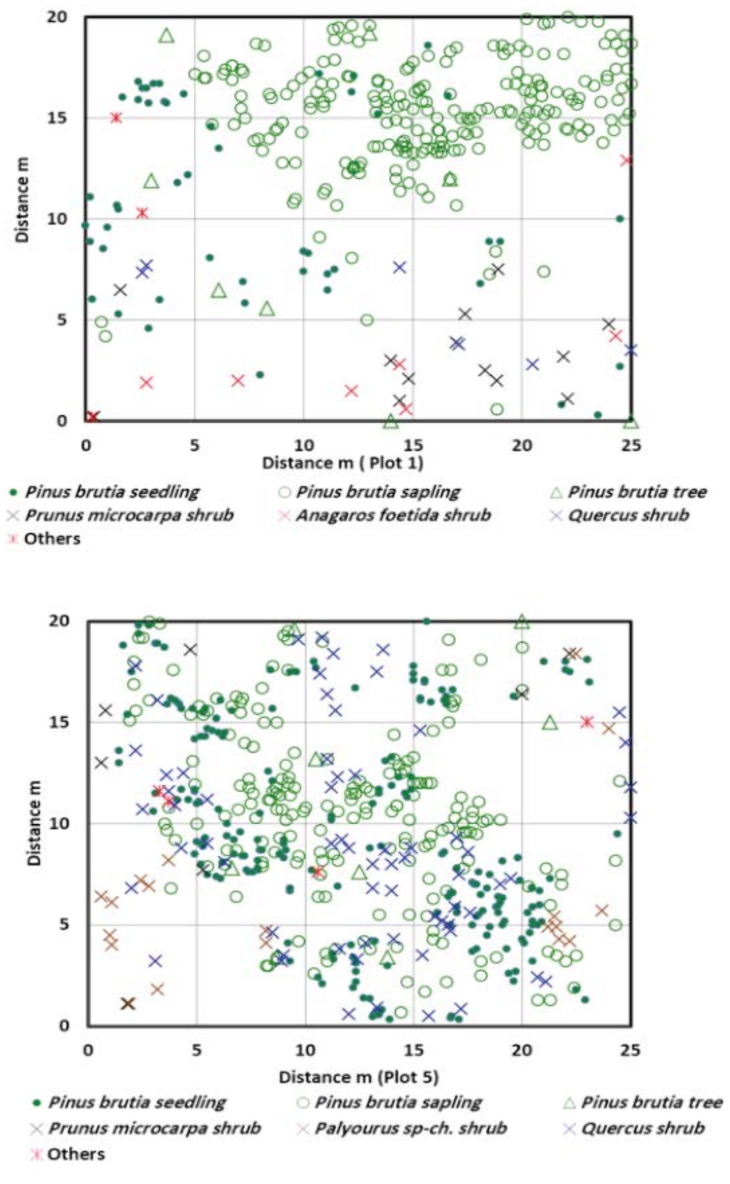

Figure 2 a, b: Examples of spatial distribution of P. brutia regeneration and the main shrub species mapped in 25 m by 20 m rectangular plots, showing the aggregation pattern of both seedling and saplings in Zawita forest

3. Results

3.1 Univariate spatial patterns of *Pinus brutia* regeneration

A total of 1024 *P. brutia* seedlings and 1039 saplings were recorded and mapped, with an average of 102 seedlings and 103 saplings per plot (Table A1). According to the univariate O11 (r) (Figures 3a, b, c, d and Table A2), the distribution pattern of seedlings and saplings of *P. brutia* in the study plots was similar. Based on the GoF tests, the regeneration displayed significant deviations from the complete spatial randomness null model. Overall, parent trees were randomly distributed up to 10 m (Table A3) while seedlings and saplings were spatially distributed in aggregation patterns within on average 2.85 m and up to m 3.8 respectively, which confirmed the visual inspection of the mapped patterns (Figures 2a, b, A2 to A8). The values of the univariate O11 (r) in this study simply mean that the observed density of *P. brutia* seedlings and saplings inside the rings with a radius of 0.25 m at the detected scales of distance was significantly greater than the density in the study plot.

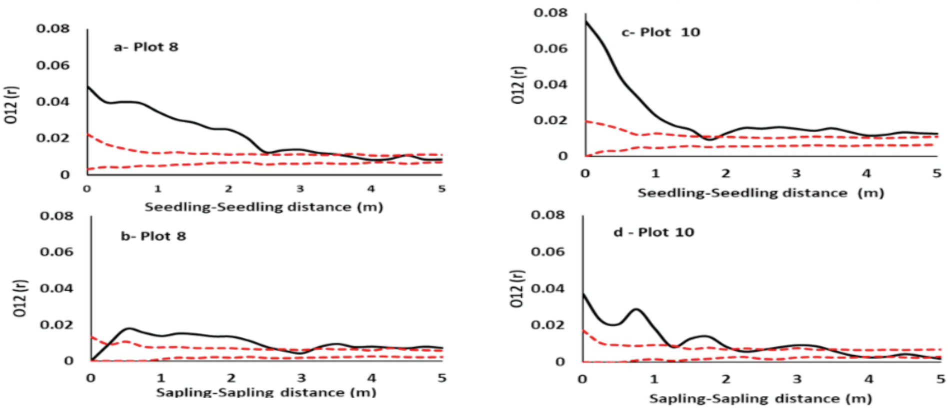

Figure 3 a, b, c, d: Examples of univariate results of the O-ring statistic for seedlings and saplings of P. brutia in two sample plots. The solid lines represent the O-ring values according to the complete spatial randomness null model, dashed lines the 95% Monte Carlo confidence intervals of the null hypothesis of complete spatial randomness. O-ring value that fall above, below and within confidence intervals indicate aggregation, regular and random spatial patterns, respectively.

Abbildung 3 a, b, c, d: Beispiele für univariate Ergebnisse der O-Ring-Statistik für Keimlinge und Jungpflanzen der P. brutia in zwei Probeflächen. Die durchgehende Linien zeigen die O-Ring-Werte gemäß dem Nullmodell der zufälligen räumlichen Verteilung und die gestrichelte Linien das 95% Monte-Carlo-Konfidenzintervallen der Nullhypothese der zufälligen räumlichen Verteilung. O-Ring-Werte, die über, unter oder innerhalb der Konfidenzinterfalle liegen, weisen respektive auf Aggregation, reguläre und zufällige Raummuster hin.

3.2. Bivariate spatial patterns of *Pinus brutia* regeneration

Analysis 1: Intraspecific spatial association

From the total of 19 bivariate parent tree – regeneration (seedlings - saplings) pair tests, the GoF tests of O12 (r) function detected significant repulsion patterns in 7 parent-seedling cases out of a potential 10, and 5 arent-sapling cases out of a possible 9, as shown in Table 1 and Figures 4a, b, c, and d. Generally, the patterns of parent tree – regeneration confirmed the existence of an intraspecific segregation pattern in the 10 study plots, however, a few cases of association were found at larger scales, in plots 4, 5, 6 (Table 1).

Table 1: Type and scale of spatial association versus distance between both seedlings and saplings of Pinus brutia with parent trees. n1 and n2 are the number of saplings, seedlings and parent trees, respectively. + indicate association, – segregation and no sign independent spatial pattern. The p-values were calculated to determine the goodness-of-fit.

Tabelle 1: Art und Ausmaß des räumlichen Zusammenhangs hinsichtlich der Distanz zwischen Keimlingen und Jungpflanzen und den Elternbäumen der Pinus brutia. n1 and n2 zeigen jeweils die Anzahl von Keimlingen, Jungpflanzen und Elternbäumen. + zeigt Assoziation, – Segregation und kein Zeichen unabhängigen räumlicher Zusammenhang. Die p-Werte wurden berechnet um die Güte des Zusammenhanges zu zeigen.

Plot	*Pinus* regeneration vs. Parent trees	n1/n2	Distance (m)																				Rank	P- value
			0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6	6.5	7	7.5	8	8.5	9	9.5	10		
Plot 1	Seedlings vs. Parent trees	49/9						+	+				-				-						94	0.07
	Saplings vs. Parent trees	224/9						-				-					-			-	-		22	0.79
Plot 2	Seedlings vs. Parent trees	82/13	-	-	-		-			-			-	-	-	-	-		-		-	-	100	0.01
	Saplings vs. Parent trees	152/13	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		100	0.01
Plot 3	Seedlings vs. Parent trees	96/6					-	-	-						-	-	-	-			-		96	0.05
	Saplings vs. Parent trees	39/6				-																	81	0.2
Plot 4	Seedlings vs. Parent trees	168/10	-	-	-	-	-		+		+				+	+	+	+		+			100	0.01
	Saplings vs. Parent trees	108/10	-		-						+	+	+	+	+	+	+	+		+			96	0.05
Plot 5	Seedlings vs. Parent trees	207/8					-	-					+	+	+			-				+	38	0.65
	Saplings vs. Parent trees	200/8									+		+				+	+	+	+			89	0.1
Plot 6	Seedlings vs. Parent trees	38/30									-	-	-	-	-	-	-	-	-	-	-		95	0.06
	Saplings vs. Parent trees	175/30	-	-			-				-	-						+	+	+	+	+	98	0.03
Plot 7	Seedlings vs. Parent trees	131/9		-	-	-	-	-	-	-	-	-	-	-	-				+		+	+	100	0.01
	Saplings vs. Parent trees	3/9	-	-	-	-	-	-	-	-	-	-	-	-	-	-							100	0.01
Plot 8	Seedlings vs. Parent trees	71/6				-	-	-								-	-						98	0.03
	Saplings vs. Parent trees	34/6						-	-	-			-	-			-						90	0.11
Plot 9	Seedlings vs. Parent trees	113/3		-	-	-	-					-		-									96	0.05
Plot 10	Seedlings vs. Parent trees	69/12		-						-										-	-	-	75	0.6
	Saplings vs. Parent trees	39/12							-	-	-	-	-	-				-					100	0.01

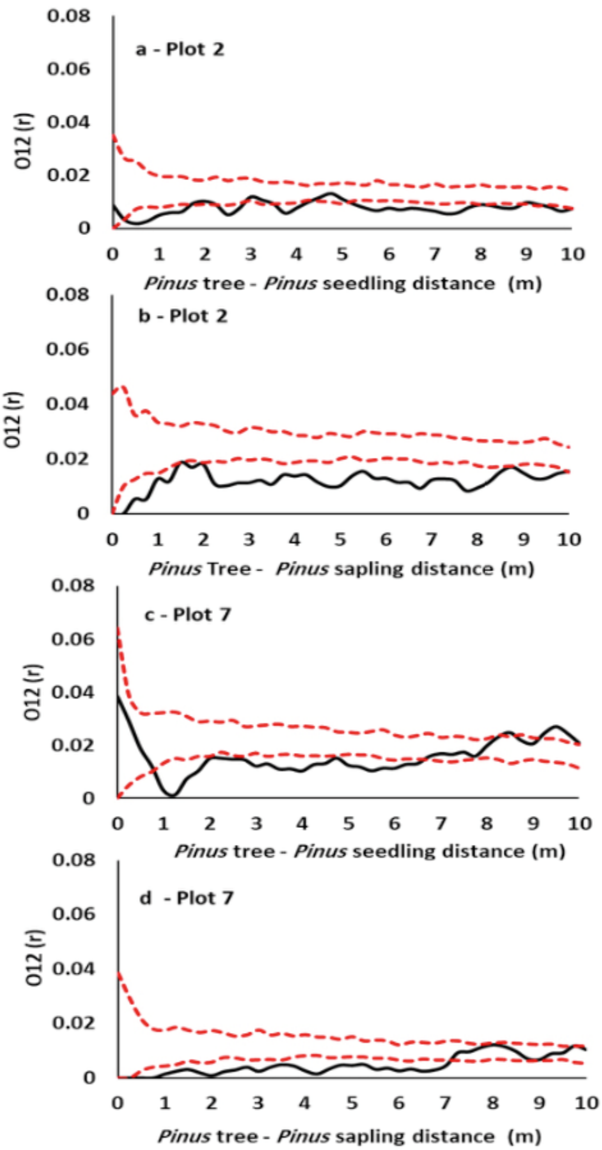

Figure 4 a, b, c, d: Examples of bivariate results of the O-ring statistic for seedlings and saplings of P. brutia Intraspecific spatial associations. For details please see Fig. 3.

Abbildung 4 a, b, c, d: Beispiele bivariater Ergebnisse der O-Ring-Statistik zur intraspezifischen räumlichen Assoziation von *P. Brutia* Keimlingen und Jungpflanzen. Für Details zur Darstellung verweisen wir auf Abb. 3.

Analysis 2: Interspecific spatial associations

The overall analysis of all 468 shrubs (ignoring their species identity) with the *P. brutia* seedlings and saplings showed that seedlings were more spatially attracted to shrubs (Table 2). GoF tests of the bivariate interspecific shrub-seedling showed 7 cases of association, 1 of segregation and 2 independent cases out of 10 cases. The distribution of shrub- sapling spatial associations was more random (7 cases out of 9) with 1 case of association and 1 of segregation (Table 2). In assessing the role of the different shrub species in the spatial association of the natural regeneration of *P. brutia*, we only accounted for the results of the analysis of *Quercus* species and *Prunus macrocarpa*, since they were only the shrub species that frequently replicated in the majority of study plots. The results of the GoF tests of shrub-regeneration spatial pattern revealed significant patterns of association between *Quercus* species and *Pinus* regeneration in 7, 4 cases and 3,4 independent patterns and 0,1 segregation pattern out of 10, 9 patterns for seedlings and saplings tested respectively (Table A4). In short, the bivariate analysis under the null model of antecedent conditions (association/segregation) between parent trees on the one hand and shrubs on the other hand with seedlings and saplings of *P. brutia*, revealed significant negative and positive spatial associations respectively. The seedlings in the 10 plots displayed an attraction to the pooled shrubs, while for the relation between saplings and shrubs no such attraction could be detected (Figure 5).

Table 2: Type and scale of association of the distance between of both seedlings and saplings of Pinus brutia with the shrubs. For details on variables see table 1.

Tabelle 2: Art und Ausmaß des räumlichen Zusammenhangs hinsichtlich der Distanz zwischen Pinus brutia Keimlingen und Jungpflanzen und Büschen. Für Details zur Darstellung verweisen wir auf Tabelle 1.

		The distance (m)										
# Plot	All shrubs	n1/n2	0.25	0.50	0.75	1.00	1.25	1.50	1.75	2.00	Rank	P- value
Plot 1	Seedlings vs. shrubs	49/28	-	+	+	+					99	0.02
	Saplings vs. shrubs	224/28	-	-	-	-	-	-	-	-	100	0.01
Plot 2	Seedlings vs. shrubs	82/39		-	-	-	-				98	0.03
	Saplings vs. shrubs	152/39								-	83	0.18
Plot 3	Seedlings vs. shrubs	96/14			+	+	+	+	+	+	100	0.01
	Saplings vs. shrubs	39/14					+		+		77	0.24
Plot 4	Seedlings vs. shrubs	168/73	+	+	+	+	+	+	+	+	100	0.01
	Saplings vs. shrubs	108/73	+	+	+	+	+	+		+	100	0.01
Plot 5	Seedlings vs. shrubs	207/96	+	+	+	+	+	+			100	0.01
	Saplings vs. shrubs	200/96									93	0.08
Plot 6	Seedlings vs. shrubs	38/26	+	+	+	+	+				100	0.01
	Saplings vs. shrubs	175/26				+	+	+			88	0.13
Plot 7	Seedlings vs. shrubs	131/23	+	+	+				+		100	0.01
	Saplings vs. shrubs	63/23									15	0.86
Plot 8	Seedlings vs. shrubs	71/60									67	0.34
	Saplings vs. shrubs	34/60					+	+	+	+	64	0.37
Plot 9	Seedlings vs. shrubs	113/67								-	66	0.35
Plot 10	Seedlings vs. shrubs	69/29		+	+	+	+				99	0.02
	Saplings vs. shrubs	39/29						+			78	0.23

Figure 5: Picture showing proximity of Pinus brutia seedlings to Quercus shrubs, where less litter and sufficient light are present.

Abbildung 5: Aufnahme von *Pinus brutia* Keimlingen neben *Quercus* Sträuchern, in deren Nähe weniger Streu und ausreichend Licht vorhanden ist.

3.3. Over- and mid-story density correlation with *Pinus brutia* regeneration

Even though we mapped all shrub species, for the analysis, we only used those whose short diameter length of the crown was at least 30 cm, expecting that when crowns were smaller than that, the shade provided by the shrubs would not be sufficient to influence the regeneration process. Generally, the presence of the parent trees adversely affected *P. brutia* seedlings. According to the results of polynomial fitting in Figure 6 and Table 3, stand basal area and the overstory canopy area exhibit a significant negatively correlation with regeneration of *P. brutia* seedlings ($R^2 = 0.65$, $P \leq 0.006$ and $R^2 = 0.75$, $P \leq 0.02$, respectively). In addition, a negative trend with the number of adult trees is evident in Table 3, indicating that regeneration of seedlings may decrease along a gradient of increasing tree density in our study plots. In contrast, the shrub density represented either by midstory canopy area or by the number of shrubs was significantly in positive correlation with *P. brutia* seedling density (Table 3). The density of *Pinus* seedlings increased with an increase in the number of shrubs and increase in the shrub crown area in the canopy ($R^2 = 0.72$, $P \leq 0.008$ and $R^2 = 0.55$, $P \leq 0.047$) respectively. In turn, no correlation was found between naturally established young saplings and the parent trees and shrub density parameters as shown respectively by the signs of the regression parameter estimates (Table 3).

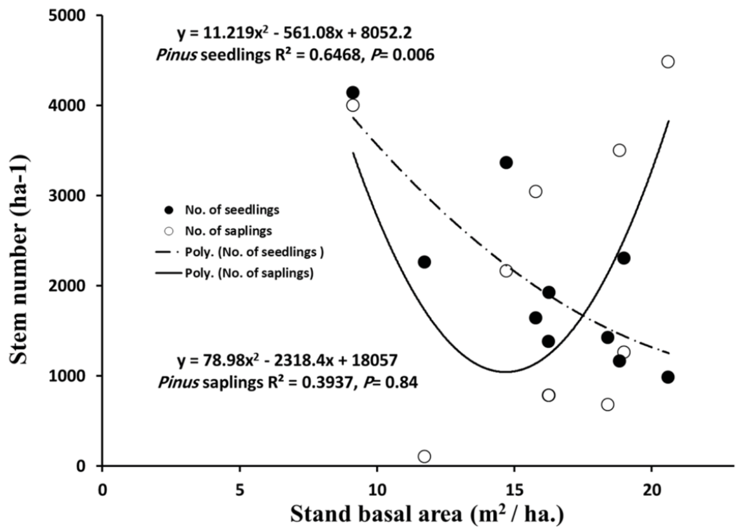

Figure 6: Second-order polynomial regression modelling the effect of stand basal area on the density of Pinus brutia regeneration.

Abbildung 6: Polynomialfunktion zweiten Grades, die Zusammenhang zwischen Baumgrundfläche und die Dichte der *Pinus brutia* Verjüngung zeigt.

Table 3: Summary of the statistical correlation between the number of the regenerated Pinus brutia (seedlings and saplings) versus stand basal area, number and crown area of trees, shrub number and crown area of shrubs.

Tabelle 3: Zusammenfassung der statistischen Korrelation zwischen der Anzahl der verjüngten *Pinus brutia* (Keimlinge und Jungpflanzen) und der Bestandesgrundfläche, der Anzahl und Kronenfläche der oberen Baumschicht sowie der Strauchanzahl und Kronenfläche von Sträuchern.

Correlation description	Model function	R^2	P-value	Type of correlation
No. *P. brutia* seedling vs. stand basal area	$y=11.219x^2-561.08x+8052.2$	0.65	0.006	Negative correlation
No. *P. brutia* sapling vs. stand basal area	$y=78.98x^2+2318.4x+18057$	0.39	0.84	Independent
No. *P. brutia* seedling vs. trees crown area	$y=0.0021x^2-1.549x+356.91$	0.75	0.020	Negative correlation
No. *P. brutia* sapling vs. trees crown area	$y=-0.0006x^2-0.1205x+117.08$	0.12	0.820	Independent
No. *P. brutia* seedling vs. shrubs crown area	$y=0.0059x^2-0.3694x+86.248$	0.55	0.047	Positive correlation
No. *P. brutia* sapling vs. shrubs crown area	$y=0.0071x^2-0.394x+68.766$	0.34	0.250	Independent
No. *P. brutia* seedling vs. No. of trees	$y=0.0076x^2-2.7612x+132.11$	0.13	0.350	Independent
No. *P. brutia* sapling vs. No. of trees	$y=0.0032x^2+3.7021x+51.186$	0.12	0.215	Independent
No. *P. brutia* seedling vs. No. of shrub	$y=0.0335x^2-2.1902x+112.82$	0.72	0.008	Positive correlation
No. *P. brutia* sapling vs. No. of shrub	$y=0.0081x^2-8.4797x+263.75$	0.30	0.810	Independent

4. Discussion

4.1. Univariate spatial distribution

Based on the Goodness-of-fit tests, compared to the obviously random distribution of parent trees, aggregated distribution was the dominant pattern approximately over all the study plots for both seedlings on average up to fine scale 2.85 m and saplings at larger scale 3.8 m, whose intensity faded gradually with increasing distance to up to 5 m. Aggregated distribution of species is a common pattern, particularly for naturally regenerated seedlings in forests (Zhang et al., 2013). Niche segregation (Pielou, 1961), habitat heterogeneity (Gholami et al., 2018), and dispersal mode (Hubbell, 2001) are considered to be the mechanisms that contribute to aggregation patterns of regeneration. In this study, the tendency of *P. brutia* to show clump distribution generally is due to the tendency of the species to regenerate in clusters in gaps avoiding the competition with parent trees for light and/or litter accumulation. However, under our study conditions, limited seed dispersal or/and biological characteristics of seeds of *P. brutia* might also explain a part of this pattern at small scales. For example, cones of coniferous trees are big and heavy, and the seeds are slightly heavier relative to their wing length (Richardson et al., 2000) leading to the shorter average dispersal distance, and then the seedlings that emerge from these seeds inevitably form aggregated distributions after germination (Seidler & Plotkin, 2006; Lin et al., 2011; Zhang, 2017). Previous studies suggested that intraspecific competition increases over tree life stages, causing changes in the aggregation distribution toward a predominant random pattern among saplings and to fit a regular pattern of the parent trees. This is because competition for resources over time will lead to mortality among individuals within the clumps, which will gradually alter the spatial distribution (Lan et al., 2012). This did not occur on the sites under examination of the present study, maybe because the saplings and the trees were not big enough to show strong competition among conspecific neighbors. In other words, *P. brutia* saplings arranged in a clumped pattern could mean that the saplings did not exhibit strong self-thinning yet, which can shift the pattern from aggregation to a random or segregation shape (Getzin et al., 2006; Yuan et al., 2018).

4.2. Bivariate spatial associations

In many cases, in severe environmental conditions, the favourable growing conditions created under the canopy of some plants appear to be the crucial factor that facilitates natural forest regeneration (Gómez-Aparicio et al., 2005; Rodríguez-García et al., 2011a; Rodríguez-García et al., 2011b; Muhamed et al., 2015). In Mediterranean ecosystems, extreme temperatures, summer drought, and irradiance are important factors affecting plant recruitment (Gómez-Aparicio et al., 2004; Puerta-Pinero et al., 2007). In this context, our expectation in this study was to find a strong spatial association pattern of *Pinus* regeneration with parent trees and neighboring shrubs. On the contrary, in almost every case of paired analysis, the *P. brutia* seedlings and saplings were found in distance from parent *P. brutia* trees, but in the vicinity of shrubs, particularly seedling-*Quercus* shrubs. These findings were confirmed by the negative correlation of both seedling and saplings with the tested density parameters of parent trees in contrast to the positive correlation with the number of shrubs and shrub crown area. This means that the microhabitat near the parent trees inhibited seedling establishment. The segregation patterns between parent trees and seedlings and between parent trees and saplings underline the significance of competition in structuring the distribution patterns of early regenerated *Pinus* individuals. These results are inconsistent with the heliophilous nature of *P. brutia* that germinates and establishes in well-illuminated places, as a pioneer and site insensitive species (Thanos & Doussi, 2000). Numerous authors have concluded that a segregation exists between the density of mature pine trees and seedlings. Palik et al. (1997) showed a negative correlation between the early seedling establishment of longleaf pine and the basal area of mature trees as a result of decreasing available light and nitrogen levels with increasing basal area. Grace and Platt (1995a, b) also detected a negative correlation between the location of seedlings with mature trees during the first year of seedling growth as a result of intraspecific competition between seedlings and the fine root biomass of mature trees. The segregation between seedlings and adult trees may also be due to increased needle litter depth around mature trees because the accumulation of litter reduces the emergence of seedlings of small-seeded tree species and prevents the radicals from reaching the soil (Grace & Platt, 1995a; Dzwonko & Gawronski, 2002; Avery, 2004). In plant-plant interactions, competition and facilitation may occur simultaneously and the net outcome of the interaction may vary both spatially and temporally (Gómez -Aparicio et al., 2005; Muhamed et al., 2015). The high number of repulsion patterns with parent trees found in this study do not exclude potential positive effects of parent trees like facilitating and sheltering the early regeneration of *Pinus* from direct radiation, but may mean that these positive aspects were outweighed by negative influences like light blocking, allelopathic, or thick litter layer under continuous canopy cover (Padilla & Pugnaire, 2006; Muhamed et al., 2018). Consequently, this may have promoted regeneration under the more favorable ecological conditions provided by shrubs compared to the conditions provided by parent trees. However, the effect of the depth of needle litter and light intensity was not within the scope of our study. It is likely that the main reasons behind the repulsion patterns are both the amount of litterfall that accumulates around the parent trees and in their understory (Muhamed et al., 2018) and the reduced availability of light that reaches the forest floor (Puerta-Piñero et al., 2007). This result is congruent with the Janzen-Connell theory explaining high offspring mortality rates near parent trees, which might also partly contribute in explaining the segregation patterns detected in this study (Matthesius et al., 2011). The spatial association pattern between shrubs and seedlings is comprehensible since shrubs like *Quercus* species with a relatively small canopy can represent a beneficial shady environment (e.g., by reducing the soil temperature and atmospheric moisture stress) and at the same time, provide sufficient light during the early establishment stages (Muhamed et al., 2018). Such results are common in Mediterranean ecosystems where pioneer shrubs can facilitate tree seedling survival and growth performance during natural establishment (Gómez-Aparicio et al., 2004). The non-significant correlation between saplings and shrubs could be due to the relatively small size of shrubs in this study compared to the well-developed root and shoot system of the saplings that help saplings continue to grow without the need for continued facilitation by shrubs. However, the shrub-seedling spatial association or the neutral shrub-sapling association may switch to segregation spatial pattern (competition) over life stage gradient, since niche requirements for the regeneration usually narrow at the seedling stage and become broader at old sapling and adult stages that may generate intensive competition with shrubs for resources over time (Quero et al., 2008).

5. Conclusion

A pattern of aggregated spatial distribution discovered in the initial stages of *P. brutia* regeneration in this study is an evidence for a weak (or absent) self-thinning process in the early stages of regeneration. Zawita pine regeneration could be facilitated by shrubs while the microhabitat close to the parent trees prevented regeneration. In this context, preserving shrub cover by forest managers, particularly of *Fagaceae*, could enhance pine recruitment in this forest. However, further research like planting seedlings under and offside the canopy of shrubs and parent trees as well as seed dispersion study should be encouraged in order to explore the effects of shrub management on the natural regeneration of *P. brutia* and allow to disentangle the respective roles of competition and limited pine seed dispersal on the natural regeneration patterns of *P. brutia*.

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Appendix

Table A1: Basic stand characteristics of the 10 study plots.

Tabelle A1: Bestandeskennzahlen der 10 Probeflächen.

Plot #	No. of *Pinus* seedlings	No. of *Pinus* saplings	Total *Pinus* regeneration	Mean of DBH cm	Stand Basal area ha/ m^2	*Pinus* trees crown area m^2	No. of *Pinus* Trees	Shrubs crown area m^2	No. of Shrubs
Plot 1	49	224	273	0.38	20.6	286.65	9	28.15	29
Plot 2	82	152	97	0.26	15.8	374.68	13	18.79	40
Plot 3	96	39	135	0.4	16.3	285.92	6	11.29	21
Plot 4	168	108	276	0.45	14.7	302.42	9	102.9	73
Plot 5	207	200	407	0.21	9.1	116.07	8	165.35	97
Plot 6	38	175	213	0.16	18.8	369.77	30	116.12	26
Plot 7	131	63	194	0.34	19.0	395.11	9	14.03	24
Plot 8	71	34	105	0.44	18.4	309.49	6	52.38	60
Plot 9	113	5	118	0.5	11.7	169.67	3	69.07	68
Plot 10	69	39	108	0.28	16.3	323.63	12	42.87	30
Total	1024	1039	1926	3.42	166.0	2933.4	105	621	468

Table A2: Type and scale of spatial distribution versus distance of both seedlings and saplings of Pinus brutia regeneration. n indicate number of plants, + aggregation pattern, - regular pattern and no sign random pattern. The p-values were calculated by goodness-of-fit (GoF) tests.

Tabelle A2: Art und Ausmaß des räumlichen Zusammenhangs hinsichtlich der Distanz zwischen Keimlingen und Jungpflanzen von Pinus brutia. n zeigen die Anzahl von Pflanzen, + zeigt Assoziation, - Segregation und kein Zeichen unabhängigen räumlicher Zusammenhang. Die p-Werte wurden berechnet um die Güte des Zusammenhanges zu zeigen.

# Plot	*Pinus* regeneration	n	Distance (m)																				Rank	P- value
			0.25	0.5	0.75	1.0	1.25	1.5	1.75	2.0	2.25	2.5	2.75	3.0	3.25	3.5	3.75	4.0	4.25	4.5	4.75	5.0		
Plot 1	Seedlings	49	+	+	+	+	+	+	+									+					100	0.01
	Saplings	224	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100	0.01
Plot 2	Seedlings	82	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+					100	0.01
	Saplings	152	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100	0.01
Plot 3	Seedlings	96	+	+	+	+	+	+	+						+								100	0.01
	Saplings	39			+	+	+	+	+			+											100	0.01
Plot 4	Seedlings	168	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100	0.01
	Saplings	108	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100	0.01
Plot 5	Seedlings	207	+	+	+	+	+	+	+	+	+	+	+	+		+		-			-	-	100	0.01
	Saplings	200	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100	0.01
Plot 6	Seedlings	38	+	+	+	+	+	+	+				+	+							-	-	100	0.01
	Saplings	175	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100	0.01
Plot 7	Seedlings	131	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	100	0.01
	Saplings	63	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			+	+	+	100	0.01
Plot 8	Seedlings	71	+	+	+	+	+	+	+	+	+	+	+	+	+	+							100	0.01
	Saplings	34		+	+	+	+	+	+	+	+	+			+	+	+	+	+	+	+	+	100	0.01
Plot 9	Seedlings	113	+	+	+	+	+	+	+	+			+			+	+	+	+				100	0.01
Plot 10	Seedlings	69	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	100	0.01
	Saplings	39	+	+	+	+		+	+	+			+	+	+			-				-	100	0.01

Table A3: Type and scale of spatial distribution versus distance of Pinus brutia parent trees. For details please see Table A2.

Tabelle A3: Art und Ausmaß des räumlichen Zusammenhangs hinsichtlich der Distanz zwischen *Pinus brutia* Elternbäumen. Für Details zur Darstellung verweisen wir auf Tabelle A2.

Plot	n1	The distance (m)																				Rank	P-value
		0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5	8.0	8.5	9.0	9.5	10.0		
Plot 1	**9**																					**15**	**0.86**
Plot 2	**13**						+	+	+	+												**79**	**0.22**
Plot 3	**6**																					**70**	**0.31**
Plot 4	**10**	+	+										+									**68**	**0.33**
Plot 5	**8**									+	+	+										**44**	**0.57**
Plot 6	**30**	+	+	+	+	+		+	+						-	+						**100**	**0.0.1**
Plot 7	**9**								+			+					+					**68**	**0.33**
Plot 8	**6**									+												**61**	**0.4**
Plot 9	**3**						+	+														**77**	**0.24**
Plot 10	**12**								+	+												**72**	**0.29**

Table A4: Type and scale of spatial distribution versus distance between both seedling and saplings of P. brutia and shrubs. For details please see Table A2.

Tabelle A4: Art und Ausmaß des räumlichen Zusammenhangs hinsichtlich der Distanz zwischen *Pinus brutia* Verjüngung und Büschen. Für Details zur Darstellung verweisen wir auf Tabelle A2.

# Plot	*Pinus* regeneration vs. Shrubs	Distance (m)									Rank	P- value
		n1/n2	0.25	0.50	0.75	1.00	1.25	1.50	1.75	2.00		
Plot 1	Seedlings vs. ***Quercus species***	49/06	+	+	+						100	0.01
	Saplings vs. ***Quercus species***	224/06		-	-	-	-	-	-	-	100	0.01
	Seedlings vs. ***Prunus microcarpa***	49/13							-		83	0.13
	Saplings vs. ***Prunus microcarpa***	224/13	-	-	-	-	-	-	-	-	100	0.01
	Seedlings vs. ***Anagyris foetida***	49/07						-	-	-	93	0.08
	Saplings vs. ***Anagyris foetida***	224/07	-	-	-	-	-	-	-	-	100	0.01
Plot 2	Seedlings vs. ***Quercus species***	82/21									81	0.2
	Saplings vs. ***Quercus species***	151/21						-	-	-	85	0.16
Plot 3	Seedlings vs. ***Quercus species***	96/5				+	+	+	+	+	100	0.01
	Saplings vs. ***Quercus species***	39/5			+				+		86	0.1
Plot 4	Seedlings vs. ***Quercus species***	168/68	+	+	+	+	+	+	+	+	100	0.01
	Saplings vs. ***Quercus species***	108/68	+	+	+	+	+		+	+	100	0.01
Plot 5	Seedlings vs. ***Quercus species***	207/66	+	+	+	+	+	+	+	+	100	0.01
	Saplings vs. ***Quercus species***	200/66			+	+	+		+	+	100	0.01
	Seedlings vs. ***Prunus microcarpa***	207/07									49	0.53
	Saplings vs. ***Prunus microcarpa***	200/07		-	-	-	-	-	-	-	100	0.01
	Seedlings vs. ***Palyourus sp-ch***	207/19		-					-	-	85	0.16
	Saplings vs. ***Palyourus sp-ch***	200/19	-	-	-	-	-	-	-	-	100	0.01
Plot 6	Seedlings vs. ***Quercus species***	38/13	+	+	+	+					100	0.01
	Saplings vs. ***Quercus species***	175/13			+	+	+	+	+	+	100	0.01
	Seedlings vs. ***Palyourus sp-ch***	38/8					+				92	0.09
	Saplings vs. ***Palyourus sp-ch***	175/8		-	-	-	-	-	-	-	100	0.01
Plot 7	Seedlings vs. ***Quercus species***	131/19				+	+	+			98	0.03
	Saplings vs. ***Quercus species***	63/19									8	0.93
Plot 8	Seedlings vs. ***Quercus species***	71/32									8	0.19
	Saplings vs. ***Quercus species***	34/32						+	+	+	94	0.07
	Seedlings vs. ***Prunus microcarpa***	71/18			-	-	-	-			97	0.04
	Saplings vs. ***Prunus microcarpa***	34/18									59	0.42
Plot 9	Seedlings vs. ***Prunus microcarpa***	113/14				-	-				66	0.35
Plot 10	Seedlings vs. ***Quercus species***	69/6	+	+	+	+	+	+			100	0.01
	Saplings vs. ***Quercus species***	39/6			+	+	+	+			98	0.03
	Seedlings vs. ***Prunus microcarpa***	69/7									61	0.4
	Saplings vs. ***Prunus microcarpa***	39/7							+		96	0.05
	Seedlings vs.***Juniperus oxycedrus***	69/8				-	-	-	-	-	99	0.02
	Saplings vs. ***Juniperus oxycedrus***	39/8			-	-	-	-	-	-	100	0.01

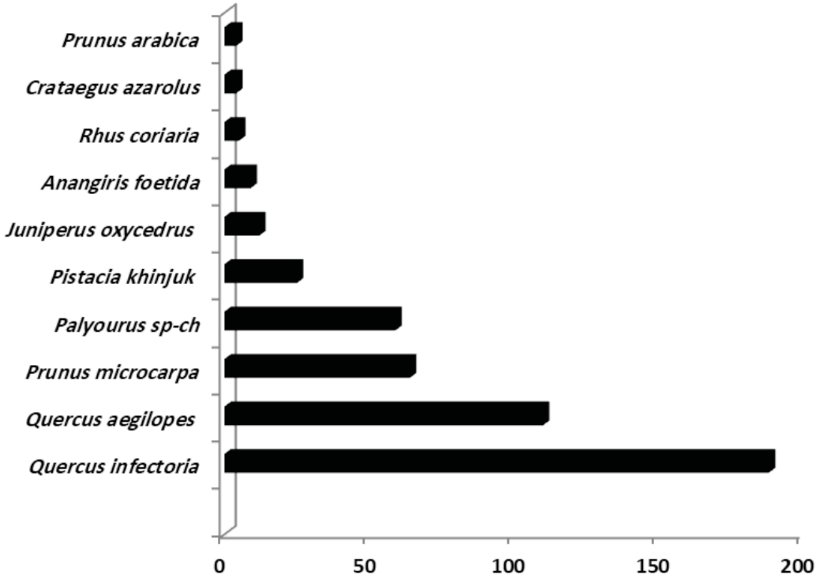
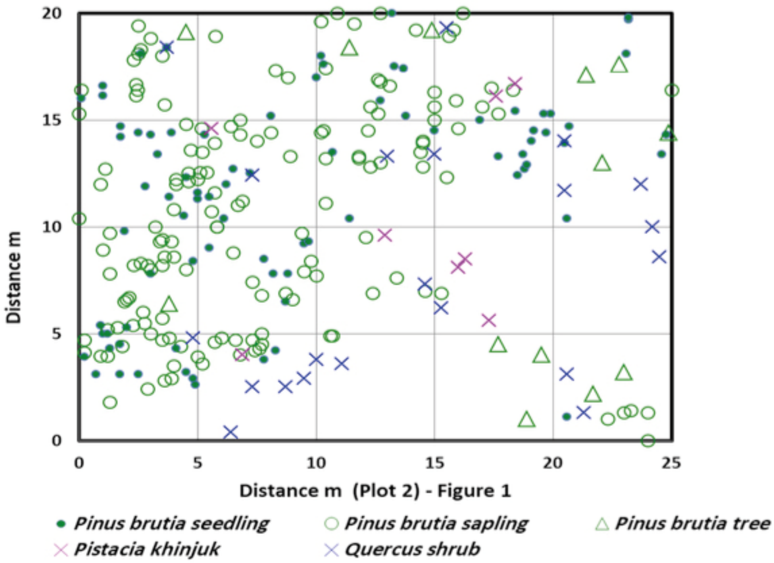


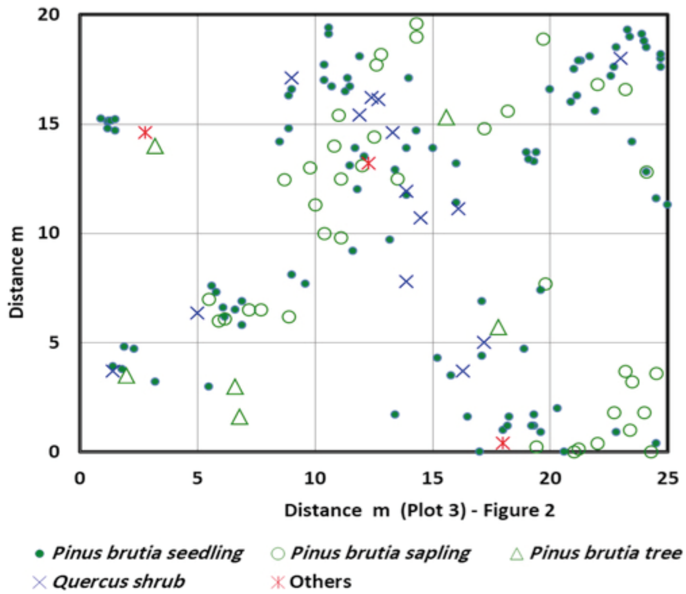
Figure A1: The main shrub species in the studied plots.

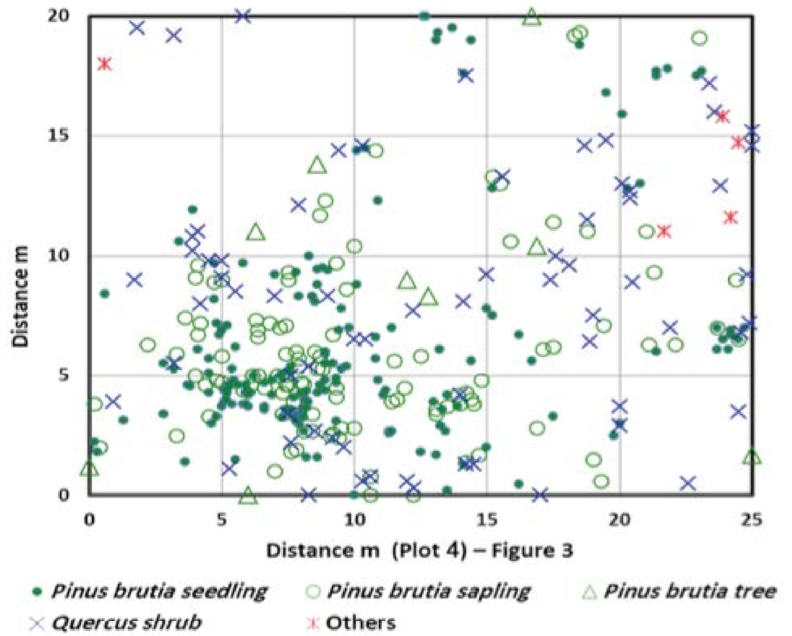
Abbildung A1: The häufigsten Straucharten auf den Probeflächen.

The following Figures A2-A8 show the spatial distribution of P. brutia regeneration and main shrub species mapped in 25 m by 20 m rectangular plots, showing the aggregation pattern of both seedling and saplings in Zawita forest.

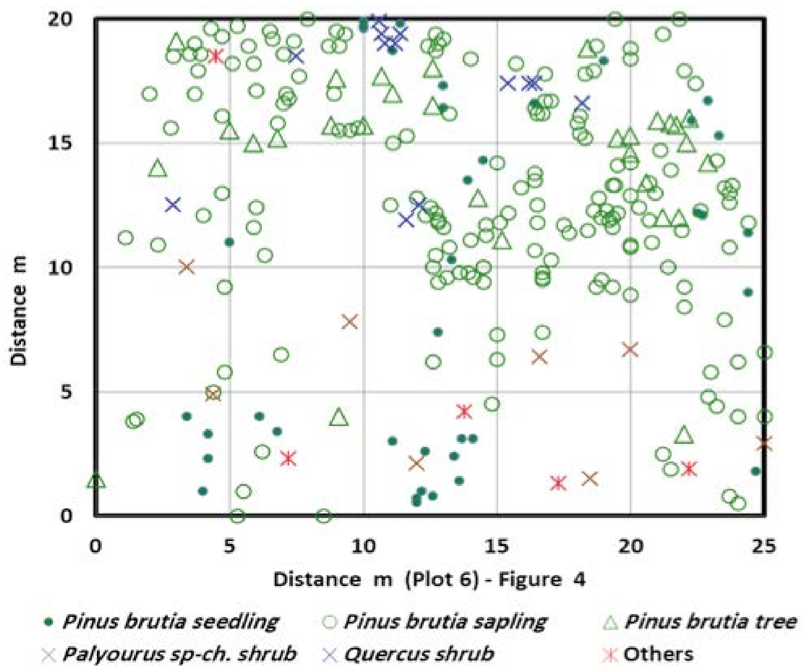
Die folgenden Abbildungen A2-A8 zeigen die räumliche Verteilung der *P. brutia*-Verjüngung und der Hauptstraucharten in 25 m * 20 m großen rechtwinkeligen Probeflächen an, die das Aggregationsmuster von Keimlingen und Jungpflanzen im Zawita-Wald aufzeigen.



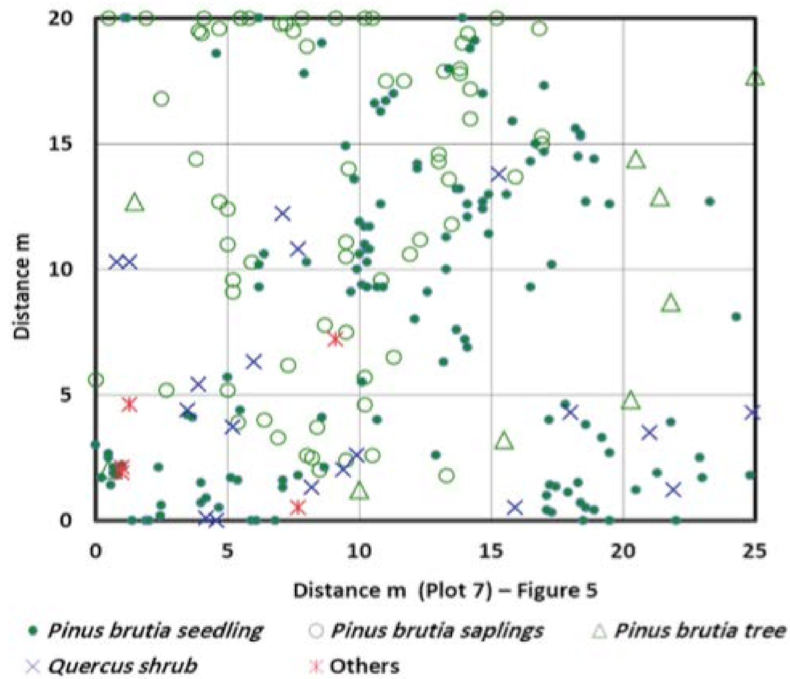


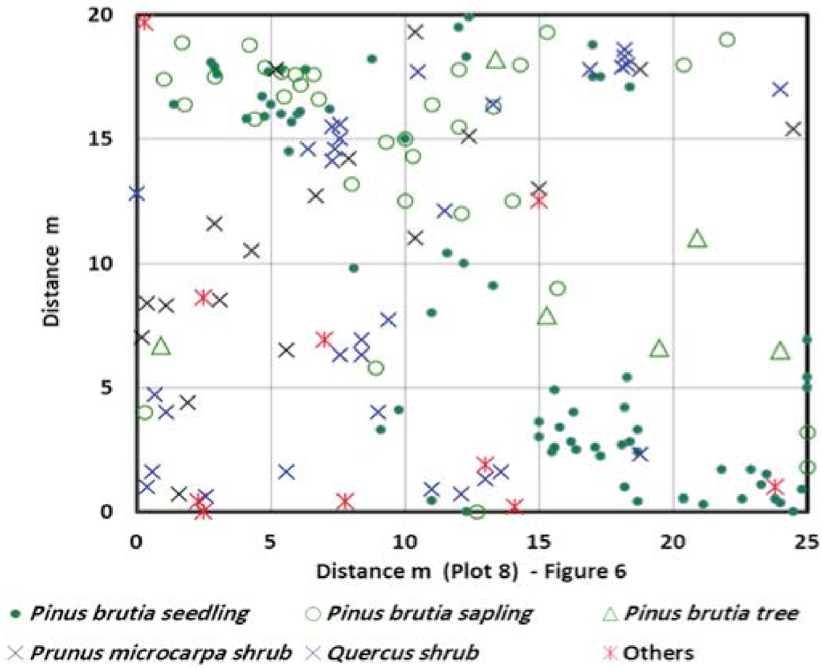


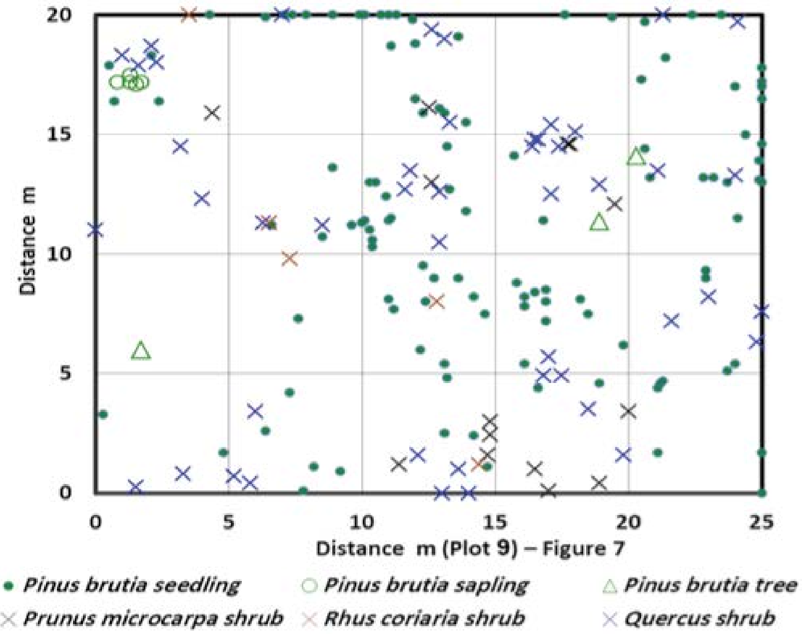
Distance m
Distance m (Plot 4) – Figure 3
Pinus brutia seedling
Pinus brutia sapling
Pinus brutia tree
Quercus shrub
Others

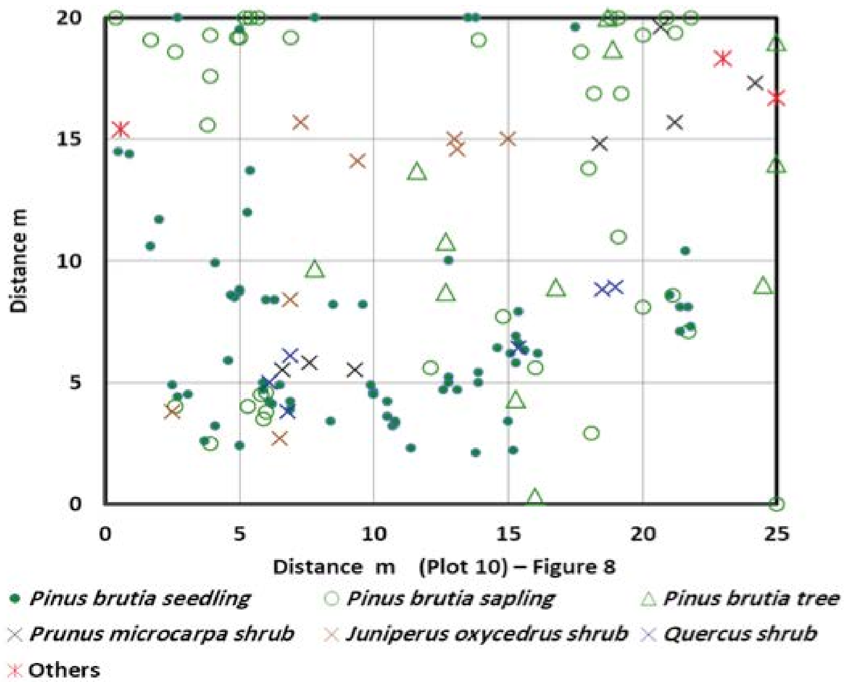


Distance m
Distance m (Plot 6) - Figure 4
Pinus brutia seedling
Pinus brutia sapling
Pinus brutia tree
Palyourus sp-ch. shrub
Quercus shrub
Others


Distance m
Distance m (Plot 7) – Figure 5
Pinus brutia seedling
Pinus brutia saplings
Pinus brutia tree
Quercus shrub
Others


Distance m
Distance m (Plot 8) - Figure 6
Pinus brutia seedling
Pinus brutia sapling
Pinus brutia tree
Prunus microcarpa shrub
Quercus shrub
Others


Distance m
Distance m (Plot 9) – Figure 7
Pinus brutia seedling
Pinus brutia sapling
Pinus brutia tree
Prunus microcarpa shrub
Rhus coriaria shrub
Quercus shrub


Distance m
Distance m (Plot 10) – Figure 8
Pinus brutia seedling
Pinus brutia sapling
Pinus brutia tree
Prunus microcarpa shrub
Juniperus oxycedrus shrub
Quercus shrub
Others