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**Black locust (*Robinia pseudoacacia* L.) in Short Rotation Systems
Stand Dynamics, Honey Potential, Invasiveness**

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‘Vegetation, “particularly trees” are highly worthy of our attention; and in itself is of the utmost consequence to mankind, and productive of many of the greatest comforts and elegancies of life.’

– **GILBERT WHITE**, *The Natural History of Selborne*, 1836



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Thesis Abstract

Various features characterize tree species, and the estimation of a tree's potential and risk is crucial for its evaluation. Black locust (*Robinia pseudoacacia* L.) can grow quickly and fix nitrogen from air. This tree species flowers intensively at a young developing stage and reproduces via seeds, stump shoots, and root suckers. However, research on the stand dynamics, honey potential, and invasiveness of black locust in short rotation systems is limited. This thesis focuses on the biomass production, growth partitioning, flower and reproduction analysis of black locust in Germany. Allometric and mixed-effects models were applied to investigate biomass production and competition. Unmanned aerial systems (UAS) were used to analyze flowering amount and spreading. Additionally, seed experiments were employed to study generative reproductive potential. Analyses showed that biomass production is mainly influenced by the tree and stump shoot diameter and height. Moreover, biomass varied significantly between former mining and agricultural areas as well as between harvested and non-harvested plantations. Phosphorus was the most important nutrient and, together with water, the main driver of growth partitioning among *R. pseudoacacia* trees. UAS and terrestrial data revealed 5.3 million flowers per hectare in an eight-year-old black locust plantation in the year 2017. These flowers provide food for many insects, especially nectar (87 kg) for honeybees (*Apis mellifera*). The amount of nectar produced by black locust flowers is sufficient for one average bee hive to survive for one year. Furthermore, honeybees could produce 70 kg of honey, which is equal to 140 honey jars (500 g). Invasiveness analysis showed that spreading increases with greater light availability and decreased tillage. Thus, the proportion and average spreading distance are high if the surrounding area is a dirt road or meadow. By contrast, the proportion and spreading distance are low if the neighboring area is a forest or farmland. Moreover, seed germination increases with increasing warm–cold variety and scarification. Overall, black locust can offer the ecosystem services of biomass production, carbon sequestration, flower and honey production (potential). Nevertheless, the varying reproduction strategies of black locust and its resulting spread to neighboring areas as risky aspect should be not negligible by evaluating this tree species.



Kurzfassung

Für die Beurteilung von Baumarten ist es wichtig die Potenziale und Risiken zu analysieren und zu diskutieren. Die Robinie (*Robinia pseudoacacia* L.) wächst schnell und besitzt die Fähigkeit Luftstickstoff zu fixieren. Zudem beginnt die Blühaktivität der Robinie sehr früh mit ca. sechs Jahren nach der Etablierung. *R. pseudoacacia* vermehrt sich über Samen, Stockausschlag und Wurzelbrut. Jedoch ist wenig bekannt über die Bestandesdynamiken, das Honigpotential und die Invasivität der Robinie in Kurzumtriebssystemen. Daher fokussiert die vorliegende Dissertation die Themenbereiche Biomasseproduktion, intraspezifische Konkurrenz, Blütenproduktion sowie die Invasivität der Robinie in Deutschland. Allometrische und gemischte (mixed-effects) Modelle werden für die Analysen der Biomasseproduktion und des Konkurrenzverhaltens angewendet. Für die Analysen der Blütenmenge und der Ausbreitung der Robinie werden unbemannte Flugsysteme (UAS - unmanned aerial systems, umgangssprachlich Drohne) verwendet. Hinzu kommen Samenexperimente, um das generative Vermehrungspotenzial zu beurteilen. Die Untersuchungen zeigen, dass die Biomasseproduktion hauptsächlich durch den Durchmesser und die Höhe des Einzelbaumes bzw. des Stockausschlages beeinflusst wird. Zudem variiert die Biomasseproduktion signifikant zwischen Bergbaufolgeflächen und ehemaligen landwirtschaftlichen Flächen sowie zwischen Kernwuchs und Stockausschlag. Die Konkurrenzanalyse zeigt, dass Phosphor der wichtigste bodenverfügbare Nährstoff ist, und zusammen mit der Wasserverfügbarkeit das intraspezifische Konkurrenzverhalten zwischen Robinien beeinflusst. Die Auswertung der UAS-Luftbilder und der terrestrischen Aufnahmen in einer achtjährigen Robinien Kurzumtriebsplantage im Jahr 2017 ergaben 5,3 Millionen Robinienblüten je Hektar. Diese Blüten dienen als Nahrungsquelle für viele Insekten. Insbesondere Honigbienen (*Apis mellifera*) profitieren von dem Nektarangebot (87 kg). Von dieser Nektarmenge könnte ein Honigbienenvolk ohne Zufütterung und Honigentnahme ein Jahr überleben. Honigbienen könnten jedoch auch 70 kg Honig erzeugen. Das wären 140 Honiggläser (500 g). Die Invasivitätsanalyse zeigt, dass die Ausbreitung der Robinie zunimmt, wenn die Lichtverfügbarkeit ansteigt und die Bodenbearbeitung reduziert wird. Daher ist der Anteil und die durchschnittliche Ausbreitungsdistanz am höchsten, wenn die Nachbarfläche eine Wiese oder ein Feldweg ist. In die Richtung zu den Nachbarflächen Wald und Feld (Ackerbau) ist die Ausbreitung geringer. Zudem steigt die Keimfähigkeit von Robiniensamen mit ansteigender Warm-Kalt Behandlung und der Verletzung der Samenschale an. Zusammenfassend zeigt sich, dass die Robinie die Ökosystemdienstleistungen Kohlenstoffspeicherung, Biomasse-, Blüten- und Honigproduktion bereitstellt (Potenzial). Jedoch sollte nicht vernachlässigt werden, dass die Robinie verschiedene Vermehrungsstrategien besitzt und sich auf angrenzende Nachbarflächen ausbreiten kann (Risiko).



I. Cumulative Thesis

I.1 Motivation

Black locust is a non-native tree species in Europe. Insertion of foreign tree species in native ecosystems is a topic often referred to in silvicultural and ecological discussions. Thereby, the focus is on the potential and risk, mainly including the biology, climate and soil adaption, **productivity, growth dynamics, intra- and interspecific competition**, silvicultural treatments, wood quality, timber processing, **biodiversity, ancillary use**, and **invasiveness**. Moreover, black locust in short rotation systems has seldom been investigated. Therefore, the current thesis deals with the stand dynamics, honey potential, and invasiveness of this species. Figure 1 shows a checklist of features that are important for tree species evaluations; check marks beside specific features show the topics analyzed and discussed in the present thesis.

<u>Checklist tree species features</u>		
	Origin and appearance	
	Biology and ecology	
	Growth and competition	✓
	Silvicultural treatments	
	Wood quality and timber processing	
	Biodiversity	✓
	Ancillary use	✓
	Risk	✓

Figure 1 Checklist of features for evaluating the potential and risk of tree species. Check marks show the topics analyzed and discussed in the present thesis.



Figure 2 provides an overview of the published original research articles that are included in the current thesis. Stand dynamics include the aboveground woody biomass and key drivers of growth partitioning. The honey potential contains a flower analysis and an estimation of the biodiversity potential. The analysis of invasiveness is used to estimate the reproduction potential and the distribution of black locust over space and time.

Black locust	Stand Dynamics	Potential/ Risk (+/-)
Ecosystem Services	Allometric Models to Predict Aboveground Woody Biomass of Black Locust (<i>Robinia pseudoacacia</i> L.) in Short Rotation Coppice in Previous Mining and Agricultural Areas in Germany	<i>Robinia pseudoacacia</i> L. Flower Analyzed by Using Unmanned Aerial Vehicle (UAV)
Competition	Key Drivers of Competition and Growth Partitioning among <i>Robinia pseudoacacia</i> L. Trees	<i>Robinia pseudoacacia</i> L. in Short Rotation Coppice: Seed and Stump Shoot Reproduction as well as UAS-based Spreading Analysis

Figure 2 Summary of the published original research articles included in the present thesis.

I.2 Research objectives of the thesis

The aim and research objectives of this thesis are

1. to develop biomass models for black locust in short rotation systems,
2. to estimate the key drivers of competition and growth partitioning among black locust trees,
3. to analyze the flower production and honey potential of black locust, and
4. to investigate the reproduction strategies and spreading of black locust in short rotation systems.



I.3 Publications

This thesis is based on investigations that were published in the following four original research articles:

1. Carl, C., Biber, P., Landgraf, D., Buras, A., Pretzsch, H. (2017a). Allometric Models to Predict Aboveground Woody Biomass of Black Locust (*Robinia pseudoacacia* L.) in Short Rotation Coppice in Previous Mining and Agricultural Areas in Germany. *Forests* 8(9), 328, doi:10.3390/f8090328, <http://www.mdpi.com/1999-4907/8/9/328>.
2. Carl, C., Biber, P., Veste, M., Landgraf, D., Pretzsch, H. (2018). Key drivers of competition and growth partitioning among *Robinia pseudoacacia* L. trees. *For Ecol Manage* 430, 86–93. <https://doi.org/10.1016/j.foreco.2018.08.002>, <https://www.sciencedirect.com/science/article/pii/S0378112718311137>.
3. Carl, C., Landgraf, D., van der Maaten-Theunissen, M., Biber, P., Pretzsch, H. (2017b). *Robinia pseudoacacia* L. Flower Analyzed by Using Unmanned Aerial Vehicle (UAV). *Remote Sens* 9(11), 1091, doi:10.3390/rs9111091, <https://www.mdpi.com/2072-4292/9/11/1091>.
4. Carl, C., Lehmann, J. R. K., Landgraf, D., Pretzsch, H. (2019). *Robinia pseudoacacia* L. in Short Rotation Coppice: Seed and Stump Shoot Reproduction as well as UAS-based Spreading Analysis. *Forests* 10(3), 235, doi:10.3390/f10030235, <https://www.mdpi.com/1999-4907/10/3/235>.

Further publication as conference paper, abstract, and article in non-international journals and websites:

- Carl, C., Biber, P., Landgraf, D., Buras, A., Pretzsch, H. (2017). Modellierung der oberirdischen Biomasse von Robinien auf Bergbaufolgefächern und landwirtschaftlichen Flächen. In: Landgraf, D. (Hrsg.) *Tagungsband 1. Erfurter Tagung Schnellwachsende Baumarten Etablierung, Management und Verwertung*, ISSN: 2567-8922; S. 66–76.
- Carl, C., Biber, P., Landgraf, D., Buras, A., Pretzsch, H. (2018). Direction Specific Radial Growth of *Robinia pseudoacacia* L. Stump Shoots – A Competition Analysis. In: Harvey, J., Lange, J., Scharnweber, T., Wilmking, M. (eds.) *Book of Abstracts. TRACE 2018 Conference*, 24-27 April 2018, Greifswald, Germany; p. 33. https://trace2018.files.wordpress.com/2018/04/book-of-abstracts_08_online-version.pdf
- Carl, C. (2018). Kurzportrait Robinie (*Robinia pseudoacacia*). [waldwissen.net](https://www.waldwissen.net/waldwirtschaft/waldbau/wuh_robinie/index_DE). https://www.waldwissen.net/waldwirtschaft/waldbau/wuh_robinie/index_DE
- Carl, C., Dinter, S., Landgraf, D., Biber, P., Buras, A., Pretzsch, H. (2018). Biomassekalkulator für Robinie in Kurzumtriebsplantagen. *AFZ-Der Wald* 12/2018, S. 14–15.
- Carl, C., Landgraf, D., Pretzsch, H. (2019). Robinie – neue Einblicke und wissenschaftliche Erkenntnisse - Biomasse, Blüte, Konkurrenz, Invasivität. In: Landgraf, D. (Hrsg.) *Tagungsband 2. Erfurter Tagung Schnellwachsende Baumarten Erntetechniken, –verfahren und Logistik*, ISSN: 2567-8922; 65–78.

In the following the summaries of the original research articles as well as the author contributions are presented. Original research articles are attached: Appendix B - Published Articles.



I.3.1 Article 1: Biomass - Allometric Equation

Carl, C., Biber, P., Landgraf, D., Buras, A., Pretzsch, H. (2017a). Allometric Models to Predict Above-ground Woody Biomass of Black Locust (*Robinia pseudoacacia* L.) in Short Rotation Coppice in Previous Mining and Agricultural Areas in Germany. *Forests*, 8(9), 328, doi:10.3390/f8090328, <http://www.mdpi.com/1999-4907/8/9/328>.

Journal Impact Factor 1.956

(<http://www.mdpi.com/journal/forests>, 07.03.2019)

Summary

Black locust is a fast-growing, nitrogen-fixating, and drought-adapted tree species that is foreign to Europe. In Germany, it was planted in short rotation systems to recultivate former brown coal mining fields and produce renewable energy as biomass. Therefore, black locust was also established in dry former agricultural areas. The basis of black locust planting is the change in energy policies toward the reduction of fossil fuel use and promotion of renewable energy. Growth and biomass development are influenced by several factors, such as genetics, soil, climate, stand density, harvesting frequency and so on. Consequently, several biomass equations and yield tables of black locust at varying stages of development in different areas exist. However, research on black locust in short rotation coppice in former mining and agricultural areas is limited. Therefore, this study aimed to provide a basis for predicting the woody biomass of black locust based on tree, competition, and site variables at fourteen sites in North-East Germany. Of the fourteen sites, nine were former agricultural sites, whereas the other five were former brown coal open-cast mining areas. The trees and stump shoots aged 1–8 years, and the study areas were characterized by a variety of climatic and soil conditions. In total, 62 sample plots with 9,729 trees were investigated. Yield data, such as root collar diameter (RCD), height (H), stand density, and weight (total wet, aliquot wet, and aliquot dry) were measured, climate data were integrated, and allometric functions were applied. Results showed the highest correlations between biomass and RCD. The best model according to the conditional coefficients of determination consisted of RCD and H as independent variables. Two competition variables (h/h_g and h/d) improved the model when assessed according to Akaike's Information Criterion. Moreover, the average biomass was 5.8 tons and 2.9 tons carbon storage $\text{ha}^{-1} \text{a}^{-1}$. Different former land uses, such as mining or agriculture, as well as growth by cores or stump shoots, significantly influenced aboveground woody biomass production. At the equal diameter trees had a higher biomass in agricultural sites and as stump shoots. In conclusion, the new biomass models developed as part of this study can be applied to calculate the aboveground woody biomass production and carbon sequestration of *R. pseudoacacia* in short rotation systems in former mining and agricultural areas.



I.3.2 Article 2: Key Drivers - Growth Partitioning

Carl, C., Biber, P., Veste, M., Landgraf, D., Pretzsch, H. (2018b). Key drivers of competition and growth partitioning among *Robinia pseudoacacia* L. trees. *For Ecol Manage* 430, 86–93. <https://doi.org/10.1016/j.foreco.2018.08.002>.

Journal Impact Factor 3.169

(<https://www.journals.elsevier.com/forest-ecology-and-management/>, 07.03.2019)

Summary

Growth partitioning among trees is influenced by the availability of above- and belowground resources and is described as the ‘mode of competition’. Estimation of the slope of the size–growth relationship in stands is one approach to analyze the influence of the supply and limitation of the main resources. Nevertheless, growth partitioning is rarely included in forest research. In particular, nitrogen-fixing *R. pseudoacacia* stands are seldom investigated, and competition among black locust trees is not understood in sufficient detail. To assess and estimate the growth partitioning among black locust trees, this study analyzed ten sites in North-East Germany with 1,333 trees consisting of 27 sample plots aged 2–32 years. The climate in the analyzed sites was similar, but the soil conditions varied. The main focus of this work is to evaluate the impact of nitrogen, phosphorus, and water supply to the growth partitioning among *R. pseudoacacia* trees. Therefore, the size–growth relationship in a mixed-effects model with a random intercept and slope was applied. Fixed effects included the basal area, phosphorus, water, sunlight competition index, and the interaction between below- and aboveground resources. The possible influences of site-specific effects of the analyzed stands were quantified with the aid of the random effects. The study shows that depending on the supply and limitation of phosphorus and water, the competition mode as well as the growth partitioning among *R. pseudoacacia* trees and stump shoots was influenced. By increasing phosphorus and water availability, the competition for aboveground resources (primarily sunlight) and the slope of the size–growth relationship increased. Large trees grew disproportionately more than smaller trees. However, if the phosphorus and water availability are decreased, the competition for the belowground resources increases, and the slope of the size–growth relationship decreases – to be more flattened. No influence on the mode of competition had the available nitrogen as belowground resource. In conclusion, phosphorus was the most important nutrient and, together with water, the main driver of growth partitioning among *R. pseudoacacia* trees.



I.3.3 Article 3: Flower Analysis - Honey Potential

Carl, C., Landgraf, D., van der Maaten-Theunissen, M., Biber, P., Pretzsch, H. (2017b). *Robinia pseudoacacia* L. Flower Analyzed by Using Unmanned Aerial Vehicle (UAV). *Remote Sens* 9(11), 1091, doi:10.3390/rs9111091, <http://www.mdpi.com/2072-4292/9/11/1091/pdf>.

Journal Impact Factor 3.406

(<http://www.mdpi.com/journal/remotesensing>, 07.03.2019)

Summary

Flowers are essential for the survival of most plant species as they are the basis of seed production. Further, there is a long-term coevolution of the flower–insect relationships. Moreover, humans benefit from honey production. Black locust begins flowering early (six years). Insects such as *Hymenoptera*, especially honeybees, benefit from this service. Nevertheless, research dealing with black locust flowers is limited. Remote sensing by satellite and airplanes is used to monitor ecosystem structures. However, detailed and specific analysis of small vegetation structures, such as flowers is difficult, particularly in complex ecosystems such as forests. Therefore, UAS enables high spatial resolution, quick turnaround times, and avoid the destruction of ecosystems. This study determined whether UAS can be used to estimate flower numbers, nectar and honey quantities, and habitat potential for honeybees of black locust. Hence, a hexacopter was flown over an eight-year-old *R. pseudoacacia* plantation in Germany during the blossoming time. The investigations were subdivided into vertical, horizontal, and flower tree analyses. There, images had been done in 13 vertical flying altitudes, as well as above a 50 m x 50 m square. Different thresholds in the blue channel were tested. Further, seven flower trees were selected and photographed by UAS, and all flowers were counted. Moreover, 500 flowers were individually photographed for 2-dimensional analysis and weighed for 3-dimensional analysis. To model the nectar and honey production, as well as the number of honeybees, information's from the literature were used. The best results were obtained when the UAS was flown as closely as possible to the tree crowns. Estimates of flower surface area ranged from 2.97% to 0.03% as the flying altitude above the crowns increased from 2.6 m to 92.6 m. The average detected flower area covered 1.33% of the total horizontal area. Flower numbers ranged from 1,913 to 15,559 per tree with an average surface area of 1.92 cm² and average volume of 5.96 cm³. The UAS monitored 11% of the total surface and 3% of the total volume. Thus, in the one-hectare black locust study area, 5.3 million flowers (yielding 70 kg of honey) were estimated; this number is sufficient for one bee hive to survive for one year. This approach was not used heretofore to estimate the flowers of black locust. Furthermore, the technique is applicable to other plant species in pure and mixed stands with highly colored flowers. Overall, UAS can be used to estimate the flower numbers, nectar and honey quantities, and habitat potential for honeybees and other insect species.



I.3.4 Article 4: Invasiveness Analysis - Risk

Carl, C., Lehmann, J. R. K., Landgraf, D., Pretzsch, H. (2019). *Robinia pseudoacacia* L. in Short Rotation Coppice: Seed and Stump Shoot Reproduction as well as UAS-based Spreading Analysis. *Forests*, 10(3), 235, doi:10.3390/f10030235, <https://www.mdpi.com/1999-4907/10/3/235>.

Journal Impact Factor 1.956

(<http://www.mdpi.com/journal/forests>, 07.03.2019)

Summary

Different reproduction strategies are an important trait that tree species need in order to both survive and to spread. Furthermore, invasiveness and distribution of species, especially of non-native ones, and the following displacement and loss of biodiversity are one of the main challenges. Analysis of the invasiveness and distribution of species is difficult, particularly in fast-changing ecosystems and environments. Moreover, black locust is able to reproduce generatively via seeds and vegetatively via stump shoots and root suckers. However, little research has been conducted on the reproduction and spreading of black locust in short rotation coppices. This research study focuses on seed germination, stump shoot resprout, and spreading by root suckering of black locust in ten short rotation coppices in Germany. Seed experiments (3,000 seeds) were done and stump shoots (5,244 stump shoots) in sample plots were analyzed. Spreading was detected and measured from UAS-based images and classification technology, specifically object-based image analysis (OBIA). In addition, the classification of single UAS images was tested by applying a convolutional neural network (CNN), a deep learning model. The seed experiments focused on six different treatments in five iterations and showed that seed germination increases with increasing warm–cold variation (23%–69%), hot water scalding (72%), and mechanical scarification (90%) of the hard and impermeable seed coat. Moreover, findings also showed that the seed germination is less than 10% when seeds are directly seeded or soaked in water (18°C) for 24 h. Furthermore, the numbers of shoots per stump decrease as shoot age increases; on average 4.2 shoots per stump one year after harvest, 3.6 shoots per stump two years after harvest, and 2.2 shoots per stump three years after harvest are observed. The spreading distance increases with increasing light availability and decreases with tillage. Thus, the proportion and average spreading distance are high if the surrounding area is a dirt road or meadow but low if the neighboring area is a forest or farmland. OBIA and CNN image analysis technologies revealed 97% and 99.5% accuracy for black locust classification in UAS images. Overall, the three reproduction strategies of black locust in short rotation coppices differ in terms of initialization, intensity, and growth performance; however, all strategies play a role in the survival, spreading, and invasiveness of black locust.



Authors contributions:

Article 1: Biomass - Allometric Equation

Christin Carl, Dirk Landgraf, and Hans Pretzsch initiated and planed the project, as well as provided the ideas and concept for the article. Christin Carl organized, conducted, ran through the data collection and experiments. Furthermore, Christin Carl calculated, analyzed, and modelled the data together with Peter Biber and Allan Buras. Moreover, Christin Carl wrote and composed the manuscript. Dirk Landgraf, Hans Pretzsch, Peter Biber, and Allan Buras contributed by revising the manuscript and rechecking the scientific advice, discussion, and editing of the language.

Article 2: Key Drivers - Growth Partitioning

Christin Carl, Hans Pretzsch, Dirk Landgraf, Maik Veste, and Peter Biber initiated and planned the project. Christin Carl organized, conducted, ran through the data collection and experiments. Furthermore, Christin Carl calculated, analyzed and modeled the data with Peter Biber. Christin Carl wrote and composed the manuscript. Dirk Landgraf, Maik Veste, Peter Biber, and Hans Pretzsch contributed by revising the manuscript.

Article 3: Flower Analysis - Honey Potential

Christin Carl, Dirk Landgraf, and Hans Pretzsch initiated and planed the project, as well as provided the ideas and concept for the article. Christin Carl organized, conducted, and ran through the data collection, experiments, calculated, and analyzed the data. Together with Peter Biber, Christin Carl modeled the data. Moreover, Christin Carl wrote and composed the manuscript. Dirk Landgraf, Hans Pretzsch, Peter Biber, and Marieke van der Maaten-Theunissen contributed by revising the manuscript.

Article 4: Invasiveness Analysis - Risk

Christin Carl, Hans Pretzsch, Dirk Landgraf, and Jan R. K. Lehmann initiated and planned the project (Conceptualization). Christin Carl and Jan R. K. Lehmann developed the methodology. Christin Carl and Jan R. K. Lehmann performed the field measurements' and experiments (Investigation). Christin Carl was the project administrator. Christin Carl analyzed the seeds, stump shoots, and images via CNN, validated and visualized the results. Jan R. K. Lehmann analyzed the spreading via OBIA. Christin Carl wrote the manuscript. Dirk Landgraf, Jan R. K. Lehmann, and Hans Pretzsch contributed by revising the manuscript. Hans Pretzsch and Dirk Landgraf supervised the project.



II. Thesis: Black locust (*Robinia pseudoacacia* L.) in Short Rotation Systems

II.1 Introduction

Tree species are characterized by varying features and offer ecosystem services to humans, animals, other plants, mushrooms, and the like. These features and services result mostly in the evaluation of the potential and risk of species. Black locust grows quickly, blooms early, and spreads widely. Nevertheless, little research has been conducted on the biomass production, growth partitioning, flower and honey potential, as well as varying reproduction strategies of black locust in short rotation systems. However, examining the details within a holistic perspective for tree species may improve the estimation of ecosystem services, potential, and risk, especially those of non-native specimens like black locust.

Black locust originates in the eastern part of North America, particularly in the Appalachian regions (LITTLE, 1971; HUNTLEY, 1990), the native range is shown in Figure 3. Today, black locust is widely distributed in Europe, Asia, Africa, Australia, and South America (MAKKAR AND BECKER, 1998; AULD ET AL., 2003; FUENTES ET AL., 2010; CIERJACKS ET AL., 2013; RÉDEI, 2013; LI ET AL., 2014; AKATOV ET AL., 2016; LEI ET AL., 2016; VÍTKOVÁ ET AL., 2017).

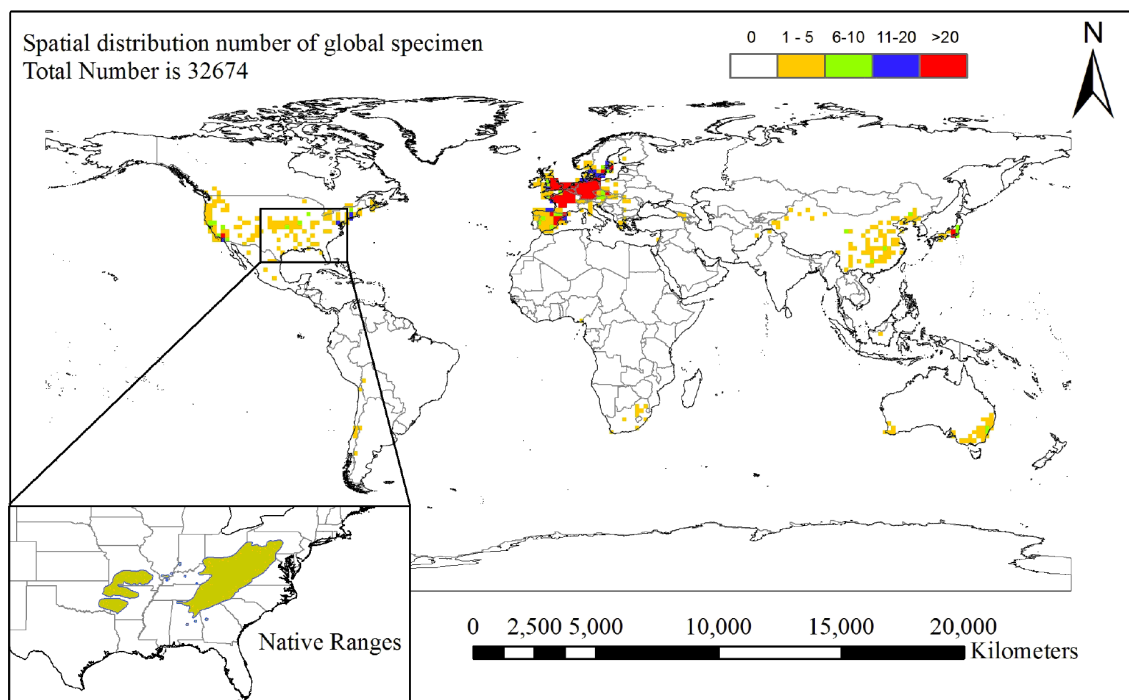


Figure 3 Global spatial abundance of black locust specimens around the world (LI ET AL., 2014) and its native range in eastern North America (LITTLE, 1971). Data: Global Biodiversity Information Facility (GBIF) and Chinese Virtual Herbarium (CVH) by LI ET AL. (2014).



In Eurasia, evidence indicates that in the past geological era “tertiary” (66–2.58 million years ago (mya), old name; today Paleogene and Neogene) *Robinia spec.* inhabited Kazakhstan (GURSKII, 1957). In the Miocene (23–5.3 mya), *Robinia* genus was found to the north of Caucasus near Amavir (GALUSHKO, 1976). Furthermore, during the Neogene (23.03–2.588 mya), *Robinia regelii* inhabited Europe as legume-type woody angiosperm (KOVAR-EDER AND KVAČEK, 2007). Thereof, *Robinia spec.* were eradicated from the Eurasian continent in the Pleistocene (2.58 mya–11 700 years ago) by repeated glaciation, similar to other tree species (KOVAR-EDER AND KVAČEK, 2007), such as *Pseudotsuga spec.* (CZAJA, 2000). However, in 1601, the foreign *Robinia pseudoacacia* L. was introduced from North America to Europe (VADAS, 1914) by the horticulturist Jean Robin. *R. pseudoacacia* was first botanically analyzed in 1753 by CARL VON LINNÉ (L.) and is named after Jean Robin (VADAS, 1914). KRAUSCH (1988) reported that *R. pseudoacacia* had been introduced to Germany in the Berliner Lustgarten in 1670. Today, black locust appears in many European countries, including Great Britain (CIERJACKS ET AL., 2013), Sweden (MOLNÁR ET AL., 2009), France and Spain (GUYOT ET AL., 1998), Italia (ANNIGHÖFER ET AL., 2012), Austria (WALTER, 2005), Switzerland (WERMELINGER AND SKUHRAVA, 2007), Germany (ERTELD, 1952), Poland (WOJDA ET AL., 2015), Czech Republic (VÍTKOVÁ ET AL., 2017), Hungary (RÉDEI, 2013), Romania (BLUJDEA ET AL., 2012) etc. In Hungary, *R. pseudoacacia* grows in 24% (460,000 ha) of the forest area (RÉDEI, 2013).

The dominant climatic conditions are an annual mean temperature of 5.8 °C to 14.5 °C and an annual precipitation of 508 to 1,867 mm (LI ET AL., 2014). Figure 4 shows the range of the annual precipitation (mm) and the average temperature (°C) by the observed presence in Europe as blue points and the field data, including absence in Europe, as grey points (SITZIA ET AL., 2016). The right-hand map shows the maximum habitat suitability in Europe (SITZIA ET AL., 2016). Black locust grows in a wide range of soil types (HUNTLEY, 1990) but generally avoids wet or compacted soils (SITZIA ET AL., 2016). VÍTKOVÁ ET AL. (2015) revealed out that black locust is able to tolerate extremely diverse chemical and physical soil conditions, from extremely acid to strongly alkaline and from medium to highly basic saturated soils with a gradient of different subsurface stoniness. On steep slopes, black locust is mildly competitive and stunted. By contrast, on sandy soils in a flat landscape, very tallest black locust trees are able to grow (VÍTKOVÁ ET AL., 2015). In the USA, *R. pseudoacacia* shows the best growth on rich, moist limestone soils (HUNTLEY, 1990). Moreover, black locust prefers well-aerated and drained soils, and tolerates desiccation but avoids compact soils and areas where the soils are frequently waterlogged (VÍTKOVÁ ET AL., 2015). DEGOMEZ AND WAGNER (2001) concluded that black locust is insensitive to a wide range of pH-values and that the best growth is obtained at neutral pH. Further, this tree species is salt tolerant.

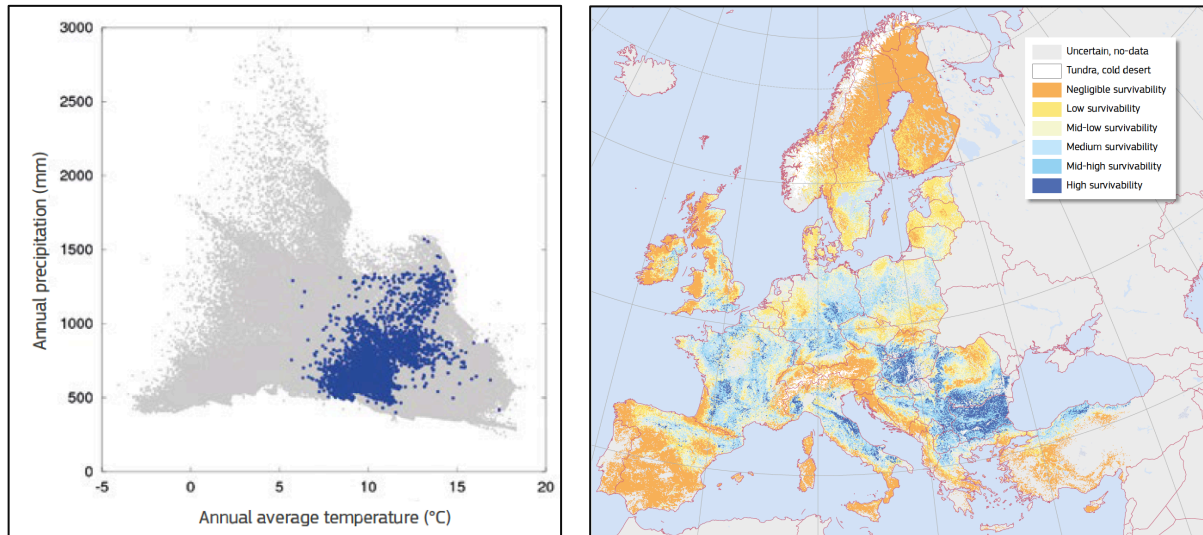


Figure 4 Black locust in Europe (SITZIA ET AL., 2016). Left: Annual precipitation (mm) and average temperature (°C) observed presence in Europe (blue points) - and field data, including absence in Europe, (gray points) by SITZIA ET AL., 2016. Right: Maximum habitat suitability in Europe by SITZIA ET AL., 2016.

MATOS ET AL. (2012) verified that the nitrogen concentration in the soil increases with increasing age of black locust stands. Moreover, LANDGRAF ET AL. (2005a) found that soils under black locust have higher contents of medium- and short-term available carbon (C) and nitrogen (N), mineral N, microbial-biomass C and N, as well as higher enzyme activities, compared with pine stands. Moreover, *R. pseudoacacia* is classified as a pioneer and catastrophe tree (HUNTLEY, 1990). All in all, black locust is limited by soil aeration, pH-value, and water supply (VÍTKOVÁ ET AL., 2015).

In Germany, the largest amount of *R. pseudoacacia* plantations are found in dry and sandy areas, such as Brandenburg, Saxony–Anhalt, Lower Saxony, Mecklenburg–Western Pomerania, along the Rhein River, and along road and train embankments. As a light-loving species *R. pseudoacacia* is often mixed with pine in northern forest stands. Moreover, in former brown-coal mining areas, *R. pseudoacacia* is often used for recultivation (GRÜNEWALDE ET AL., 2009). Additionally, as a salt-tolerant species, *R. pseudoacacia* is also often established in cities (MOSER ET AL., 2017). Furthermore, black locust is also planted in short rotation coppices. Historically, coppice stands were very common in the European countryside until the advent of fossil fuels in the 19th century (HÉDL ET AL., 2010). Thereafter, interest in coppice management has declined, leading to abandonment and conversion into high forest (HASEL AND SCHWARTZ, 2006). However, in Europe, particularly, Germany, a change in energy policies toward reductions in fossil fuel use and increases in renewable energy use, as outlined in the “Erneuerbare Energien Gesetz” (EEG 2004) (WÜSTENHAGEN AND BILHARZ, 2006), marked an increase in fast-growing tree production. Thus, in the context of the increasing support and use of renewable energies, in dry agricultural sites and former mining sites, land owners and companies (LANDGRAF AND BÖCKER, 2009; LANDGRAF AND WINKELMANN, 2010; ENERGY CROPS GMBH, 2018) began to plant *R. pseudoacacia* in agroforestry systems (BÖHM, 2016) and short rotation coppices.



Depending on the management objectives the rotation cycle may be as 2–3 (short), 4–10 (medium), or 10–20 years (long) (BIELEFELDT ET AL., 2008; BEMMANN AND BUTLER MANNING, 2013). To initiate short rotation coppice, black locust seedlings are typically cultivated for 1–2 years in tree nurseries and then grown in plantations. All in all, black locust possesses many growth characteristics that make it ideal for short rotation biomass production, such as rapid growth, high drought tolerance, and nitrogen fixation (HOFFMANN, 1961, 1964), and the ability to reproduce via stump shoots in response to harvest. Furthermore, black locust is a deciduous, intensively flowering, as well as generative and vegetative reproductive tree species.

II.2 Stand dynamics: biomass production and competition

II.2.1 Introduction

Young trees, stump shoots, and root suckers of black locust grew rapidly. Within the first year of growth, non-harvested trees can reach an average height of 1.8 m and a maximum height of 2.2 m (MEYER-MÜNZER ET AL., 2015). Moreover, stump shoots can reach 4.9 m within the first year of growth (LANDGRAF ET AL., 2005b; LANDGRAF ET AL., 2007). After the juvenile growing period, however, growth occurs very slowly. The average lifespan is 100 years (ROLOFF, 2015). Black locust is a medium-sized tree, with a height of 12–18 m and diameter of 30–76 cm. ROACH (1965) described that on better sites it may grow to a height of 30.0 m and a diameter of 122.0 cm. Many studies have dealt with black locust yield tables, growth and biomass equations (Appendix A – A. Supplementary Material). Most previous investigations of black locust biomass were published in the USA, Germany, and Hungary. These studies concentrated on forest stands and plantations. Yield tables for Germany were published in 1952 by ERTELD and in 2015 by LOCKOW AND LOCKOW. LOCKOW AND LOCKOW (2015) showed that the best black locust stands reach an average height of 36.5 m, an average diameter of 49.0 cm, and a total growth production of stump wood (Gesamtwuchsleistung – GWL Schaftholz) of 935 m³ per hectare over a period of 100 years (0.5 yield class). In the yield class 4.5, the stands show an average height of 20.4 m, an average diameter of 23.0 cm, and a total growth production of stump wood 373 m³ per hectare over a period of 100 years. The diameter of yield class 4.5 is half the diameter of yield class 0.5, which indicates that the growth depends on the environment and soil conditions.

Woody biomass is a very important renewable resource that can be used for building purposes and energy demands. Black locust wood differs among juvenile and adult trees (DÜNISCH ET AL., 2007; KOCH AND DÜNISCH, 2008; DÜNISCH ET AL., 2010; LATORRACA ET AL., 2011). Adult black locust wood is hard and has a long lifetime (adult heartwood permanency 1–2: durable to strongly durable) (Dauerhaftigkeitsklasse 1–2, DIN EN 350:2016-12). The heartwood is characterized by the presence of dark yellow-brown extractives, which give the wood its distinct color and decay resistance.



The shrinkage (radial and tangential) of the wood increases with progressive hot-water extraction (ADAMOPOULOS, 2002). Black locust wood is processed as saw logs, playground devices, working tools, piles (wine), underground mining timber, and firewood, among others (ROLOFF ET AL., 2010; FINDEISEN, 2017). Moreover, DITTRICH (2017) showed that producing veneers of black locust with a high quality is possible. However, for OSB (oriented strand/ structural board) production, black locust wood presents insufficient strand quality. Thereby, earlywood has very low strength due to its microanatomical structure, which results in disadvantageous splinters (TREML AND JESKE, 2012).

Nevertheless, the objectives of black locust planted in short rotation systems are mainly the use as energy wood. After harvest, wood chips are produced and burned to produce energy (SCHWEIER, 2013). To calculate the amount of energy produced by black locust wood, biomass models are crucial. Several biomass equations of black locust have been published, and some of these equations are shown in Figure 5. There are high differences among the correlations of the diameter and biomass. However, no allometric equation is yet available to calculate the woody biomass in short-rotation systems planted in previous mining and agricultural areas under the influence of varying age, site, and growth conditions.

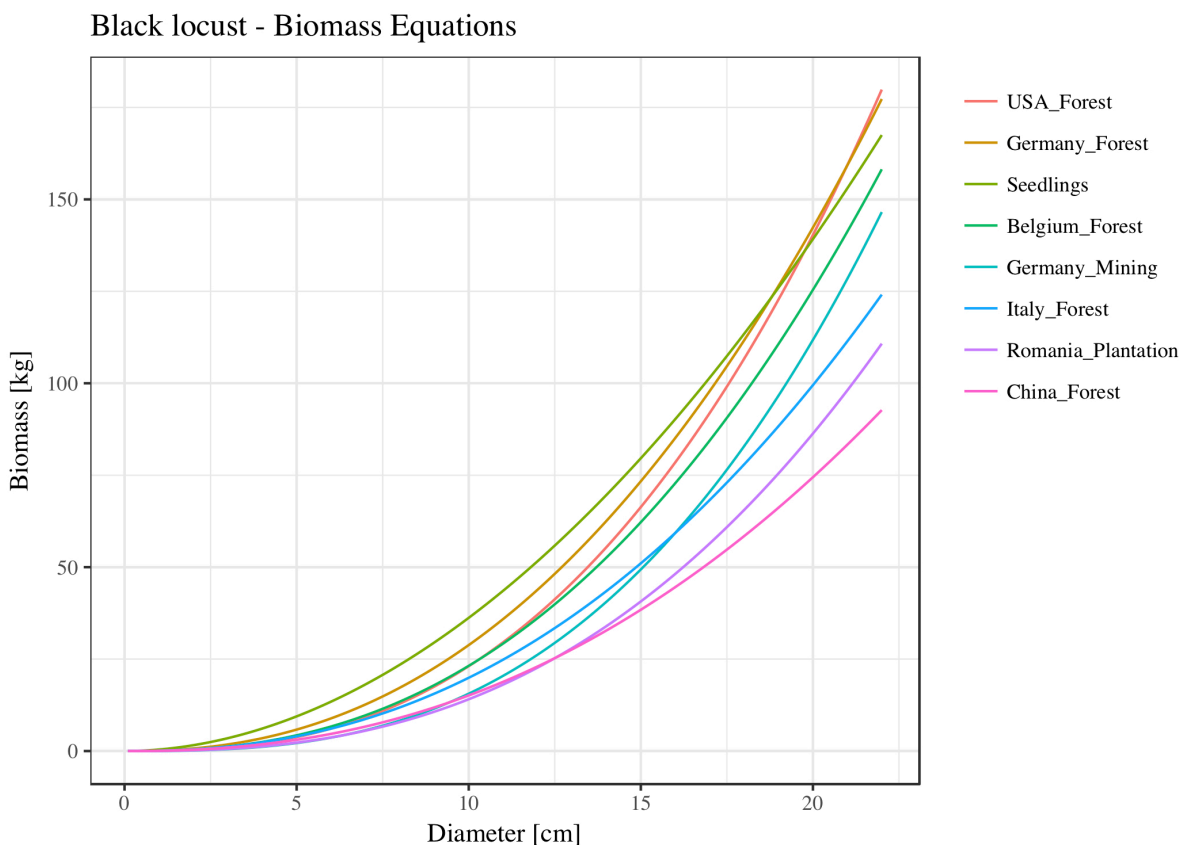


Figure 5 Biomass equations of black locust published globally. This overview is not exhaustive: USA_Forest = ELLIOTT ET AL., 2002; Germany_Forest = ENGEL ET AL., 2015; Seedlings = ANNIGHÖFER ET AL., 2016; Belgium_Forest = VAN NEVEL ET AL., 2011; Germany_Mining = BÖHM ET AL., 2011; Italy_Forest = ANNIGHÖFER ET AL., 2012; Romania_Plantation = BLUJDEA ET AL., 2012; China_Forest = LI AND LIU, 2014.



Hence, the first research article (CARL ET AL., 2017a) addresses this research gap. The objectives were (1) to develop allometric biomass equations for black locust growth in short rotation systems in Germany; (2) to assess whether inclusion of competition and climate variables improves the predictions of these allometric biomass equations; (3) to determine whether different biomass equations are necessary for trees grown in former agricultural and mining sites, as well as trees grown from the core versus stump shoot; and (4) to estimate the absolute woody biomass production and carbon storage per hectare and per year of black locust in the analyzed study stands.

Biomass equations estimate the biomass per tree and are mostly used as basis to calculate the biomass of a stand using an average value and the number of trees per hectare. However, growth per individual tree is strongly influenced by environmental factors and competition, such as self-thinning or adaptation to density as stand dynamics (PRETZSCH, 2009). Therefore, the focus of the second research article (CARL ET AL., 2018) was to find key drivers of competition among black locust trees. Competition refers to the interaction of a minimum of two individuals of one species (intraspecific) or among different species (interspecific) for resources and space. The size–growth relationship (Figure 6) is one approach to analyze growth partitioning among trees in stands depending on their individual sizes.

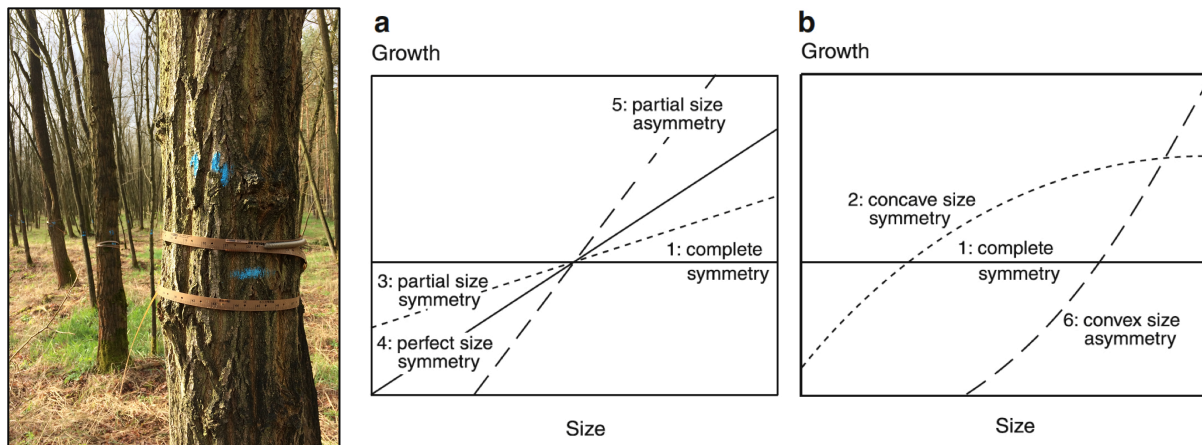


Figure 6 Permanent tree girth band D1 (UMS GMBH, 2017) in Calau, Brandenburg, Germany, 23.04.2017 (left). Depiction of the size–growth relationship between tree size and absolute growth rate, depending on above- and belowground resources (right). (a) Different linear and (b) nonlinear relationships between size and growth (PRETZSCH AND BIBER, 2010; PRETZSCH AND DIELER, 2011; PRETZSCH ET AL., 2012). Line 1 represents completely symmetric size–growth relationship in which all trees received the same resources and growth partitioning irrespective of size (more theoretical case). Line 2 reflects nonlinear concave size symmetry where growth increases less (degressively) as size increases. Line 3 displays partial size symmetry in which growth increases linearly with size. Line 4 stands for perfect size symmetry, where growth increases proportionally with size. Line 5 represents partial size asymmetry where growth increases disproportionately with size. Line 6 displays nonlinear convex size asymmetry as the growth increases (progressively) as size increases (PRETZSCH ET AL., 2012).



The slope of the size–growth relationship is mainly influenced by the supply and limitation of above- and belowground resources (WEINER AND THOMAS, 1986; WEINER, 1990). An increasing slope of the size–growth relationship (more asymmetric) indicates the increasing availability of belowground resources, consequently, increasing competition for aboveground resources, primarily sunlight (WEINER, 1990). Sunlight and resource partitioning is disproportionately available to larger trees, which shade smaller trees (FORD, 1975; CANNELL AND GRACE, 1993; WEINER AND THOMAS, 1986; SCHWINNING AND WEINER, 1998). By contrast, a decreasing slope (more symmetric) indicates increasing competition for belowground resources, primarily nutrients and water (WEINER, 1990; KRANNITZ ET AL., 1991; SCHWINNING AND WEINER, 1998). Irrespective of size, trees receive nearly the same resources and growth partitioning (PRETZSCH ET AL., 2012).

Moreover, *R. pseudoacacia* (family *Fabaceae*) is a tree species that is able to compensate for limited available soil nitrogen. With the symbiotic aid of rhizobia, black locust is capable of fixing nitrogen from the atmosphere (HOFFMANN, 1961, 1964; VESTE AND KRIEBITZSCH, 2013). However, detailed information about nutritional allocation and growth partitioning among black locust trees are not available. CARL ET AL. (2018) studied the intraspecific competition of *R. pseudoacacia* by investigating the competition for above- and belowground resources among different sized trees. The objective was to analyze whether the supply of nitrogen, phosphorus, and water as belowground resources impact the competition mode of *R. pseudoacacia* by varying growth partitioning.



II.2.2 Material and Methods

II.2.2.1 Study Sites

The study sites analyzed in this thesis and in the related original research articles are presented in Figure 7 and Table 1. The research focused on twenty sample sites with 11,091 black locust trees and stump shoots in Brandenburg, Saxony-Anhalt, and Mecklenburg-Western Pomerania (North-East Germany) with ages of 1–32 years.

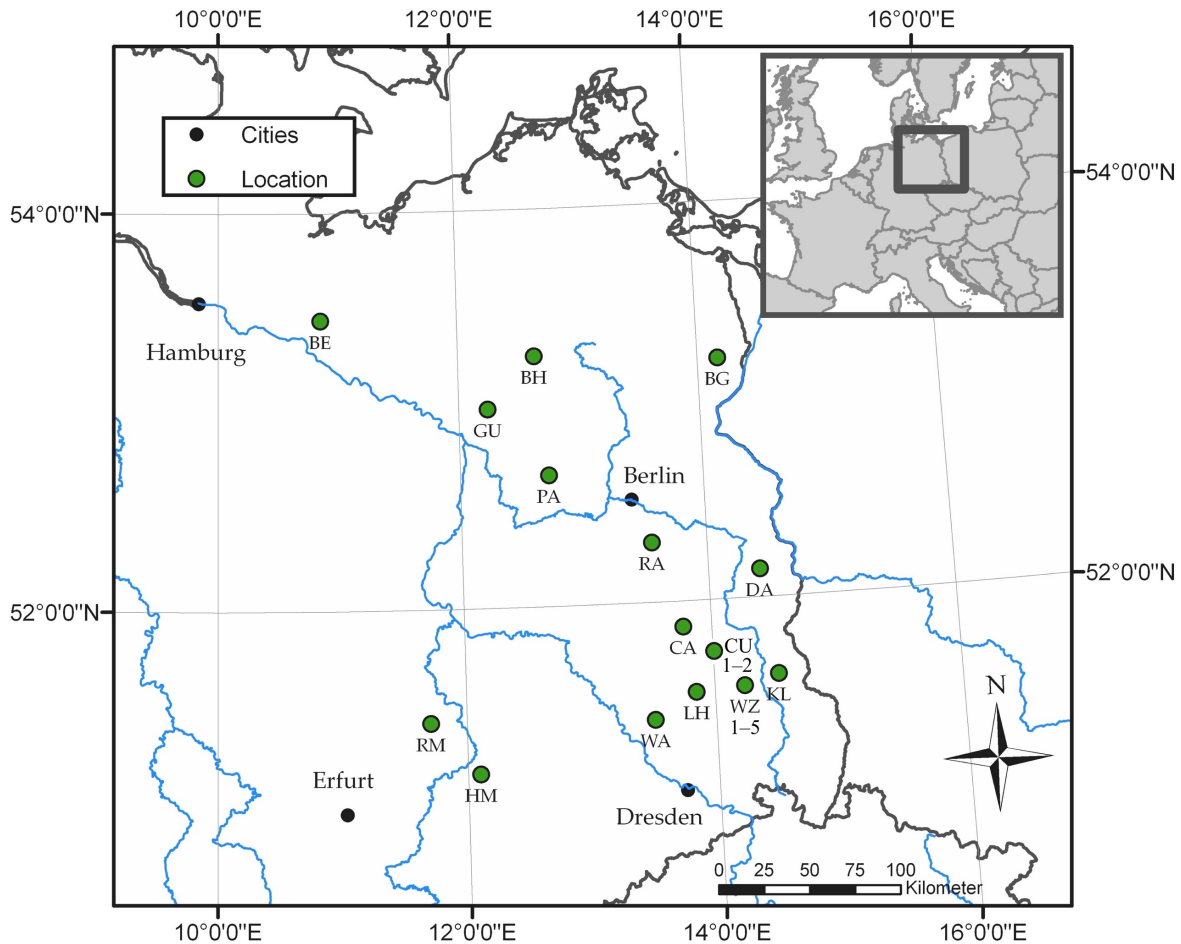


Figure 7 Map of the twenty sampling sites (locations) of *Robinia pseudoacacia* L. in Europe, especially in Germany. Site abbreviations are provided in Table 1.

Thereby, fourteen sites for biomass analysis, ten sites for competition analysis, seven sites for stump shoot analysis, two sites for seed collection, one site for flower and for invasiveness analysis were included. This work focuses mainly on short rotation systems. Among the sites targeted, nine sites are former agricultural sites that had been used for former arable farming. Further nine sites are reclaimed post-mining sites in which lignite open cast-mining activities had been carried. Moreover, two stands included in the competition analysis are forest stands. Thereby, the age in the year 2017 is considered the tree age after planting or stump shoot age since the last harvesting. Further site details are available in the original research articles.



Table 1 Site description, including locations, federal states: BB = Brandenburg, ST = Saxony-Anhalt, MV = Mecklenburg-Western Pomerania, topic: BM = Biomass, Cp = Competition, S = Stump Shoots, F = Flower Analysis, I = Invasiveness, Sc = Seed collection, longitude (long), latitude (lat), former utilization, and age in the year 2017. Further site details are presented in the original research articles.

Site	Location	Federal states	Topic	Long [°E]	Lat [°N]	Former utilization	Age [years]
BE	Bennin	MV	BM	10°51'49	53°27'45	Agriculture	8
BG	Blumberg	BB	BM + S	14°10'24	53°12'25	Agriculture	3
BH	Buchholz	MV	BM + S	12°38'9	53°15'34	Agriculture	2
CA	Cahnsdorf	BB	BM + C	13°45'54	51°52'18	Agriculture	5
CU1	Calau Mlode	BB	Cp	13°58'12	51°46'52	Forest	32
CU2	Calau Sperr	BB	Cp	13°51'57	51°47'15	Forest	22
DA	Dammendorf	BB	BM + I	14°25'5	52° 8'26	Agriculture	3
GU	Gumtow	BB	BM + S	12°14'11	52°59'46	Agriculture	2
HM	Hohenmölsen	ST	BM	12° 6'1	51°10'23	Mining	2
KL	Klein Loitz	BB	BM + S	14°30'57	51°36'35	Agriculture	2
LH	Lauchhammer	BB	BM + F + Cp + Sc	13°50'57	51°32'20	Mining	8
PA	Paulinenaue	BB	BM + S	12°43'52	52°39'44	Agriculture	3
RA	Ragow	BB	BM	13°33'7	52°18'8	Mining	7
RM	Röbblingen	ST	BM + Sc	11°42'42	51°25'57	Mining	7
WA	Wainsdorf	BB	BM + Cp + S	13°29'40	51°24'50	Agriculture	1
WZ1	Welzow EEW	BB	BM + Cp + S	14°14'7	51°33'32	Mining	1
WZ2	Welzow HW	BB	Cp	14°16'00	51°36'09	Mining	9
WZ3	Welzow KA	BB	Cp	14°16'50	51°36'06	Mining	12
WZ4	Welzow N	BB	Cp	14°19'19	51°37'15	Mining	7
WZ5	Welzow 95er	BB	Cp	14°17'55	51°34'43	Mining	22

II.2.2.2 Biomass - Allometric models

For biomass analysis, fourteen sites (62 sample plots) with 9,729 black locust trees were investigated in the winter of 2016/17 (Table 1–Topic: BM). The sites differed in age, former utilization, climate, harvesting period, rotation, and planting space. Thereby, biomass analysis focused on the correlations and allometric relationships between the biomass (Figure 8) and tree variables (e.g. root collar diameter (*RCD*), tree height (*H*)), competition and climate variables (DWD, 2017). Pearson correlation matrix (PEARSON, 1901) was applied. Furthermore, linear regression models were calculated to test the structure of individual tree data. However, linear models do not represent the real nature of biomass production. The correlations between the growth variables of single trees can be described by an allometric function. Furthermore, all regressions were undertaken using natural logarithmic transformation. As final model a nonlinear least square regression was employed to obtain estimates of the coefficients *a* and *b*. A mixed model (Equation 1) allows the use of different data frames within one model:

$$\ln(y)_{ijk} = a_0 + a_1 \ln(x)_{ijk} + b_i + b_{ij} + \varepsilon_{ijk} \quad (1)$$

Here, *x* represents tree features, competitive indices, and climate parameters (altogether, 17 variables were tested). The three different subdivisions are location (*i*), circle of the samples (*j*), and the individual trees (*k*). To quantify model performance and to compare among models, an analysis of variance (ANOVA) was performed.



The standard error was applied as the standard deviation of the sample distribution, and p -values were derived. Conditional coefficients of determination (cR^2) (NAKAGAWA AND SCHIELZETH, 2013), Akaike's Information Criterion (AIC) (AKAIKE, 1973, YAMAOKA ET AL., 1978), and the variance of the residuals were used to select and rank the best fitting models.



Figure 8 *R. pseudoacacia* short rotation coppice in Lauchhammer, a former mining site. Core growth of eight-year-old *R. pseudoacacia* with leaves and seeds, 15.09.2016 (left) and a former agricultural area called Blumberg, Brandenburg, three-year-old stump shoots, 09.01.2017 (middle). Laboratory of the University of Applied Science in Erfurt, Faculty Landscape Architecture, Horticulture und Forestry (right). Biomass samples of *R. pseudoacacia* before and after drying and weighing, 05.02.2017 (photos: Christin Carl).

To compare and cross-check the influence of different sites and growth conditions, dummy variables (a_3 and a_4) were used (Equation 2). Two different options for different growth exist in the data frame: former utilization (mining vs. agriculture) and type of growth (core vs. stump).

$$\ln(BM) = a_1 + a_2 \ln(RCD) + a_3x + a_4 \ln(RCD)x \quad (2)$$

To estimate the total woody biomass production and carbon storage, the dry woody biomass per sample plot was calculated, projected to one hectare, and divided by age. Following IPCC (2003), the dry woody biomass estimates were multiplied by 0.5 to estimate the carbon stock. Moreover, specific biomass equations for varying conditions were developed and inserted in an application (App) to calculate the biomass production of *R. pseudoacacia* in short rotation systems in former mining and agricultural areas, as well as that during core and stump growth. The biomass calculator can be accessed via the following link: <https://www.fh-erfurt.de/pub/BiomassekalkulatorRobinie/>.

II.2.2.3 Competition - Mixed-effects models

While focusing on the above- and belowground competition, it was important that the climate region is similar. Thus, the ten stands (27 sample plots) were spatially close (Table 1, Topic: Cp) and included 1,333 black locust trees. For the growth partitioning analysis, the main focus was on the growth. Hence, with the aid of a measurement tape and girth band D1 (UMS GMBH, 2017), the diameter at breast height (DBH) growth was measured in spring 2017 before leaf proliferation and in autumn 2017 after the annual growth period (Figure 6).



Additionally, stand structures, such as coordinates (x and y values), tree height, crown base, crown radius (north, south, west, and east) were measured, basal area (BA) and sunlight competition index (CI_{Light}) (PRETZSCH ET AL., 2002; PRETZSCH AND BIBER, 2010) were calculated, and the soil texture and nutrients till one meter depth were analyzed in the laboratory. The size–growth relationship (Figure 6) was described in a mixed-effects model (ZUUR ET AL., 2009) with a random intercept and slope (Equation 3):

$$\ln(gBA_{ij}) = b_0 + b_1 \ln(BA_{ij}) + b_2(CI_{Light_{ij}}) + b_3(P_{ij}) + b_4(AWC_{r_{ij}}) + b_5(CI_{Light} * P)_{ij} + b_6(CI_{Light} * AWC_r)_{ij} + c_i + d_i * \ln(BA_{ij}) + \varepsilon_{ij} \quad (3)$$

The BA and BA growth (gBA) in a natural logarithmic transformation were applied, caused by their high homoscedasticity compared with DBH and DBH growth. Moreover, the response variable is gBA_{ij} , which refers to the gBA of tree j on site i , and the corresponding explanatory variables are BA , CI_{Light} , phosphorus (P), available water capacity of the roots (AWC_r), and the interactions among $CI_{Light} * P$ and $CI_{Light} * AWC_r$ of tree j on site i . The fixed term in Equation 3 contains all explanatory variables with the coefficients b_0 – b_6 . For the random term, the random intercept c_i per site i and the random slope as $d_i * \ln(BA_{ij})$ per site i are used. Thereby, random effects show the possible influences of further unknown site-specific growth effects independent of the chosen explanatory variables (ZUUR ET AL., 2009). Moreover, ε_{ij} represents the independent observation error. The software R, version 3.3.2 (R CORE TEAM, 2016), and the listed application packages were used for all statistical and graphical processes in the original research articles CARL ET AL. (2017a) and CARL ET AL. (2018).



II.2.3 Results

II.2.3.1 Biomass

The Pearson correlation matrix (Table 2) shows the correlations among all included tree and stand variables. Low correlations among biomass dry (*BMD*) and climate variables (precipitation, temperature, sun duration, De Martonne index, and climatic water balance) were obtained. The strongest correlation to *BMD* showed the variables *RCD*, *DBH*, *H*, *Age*, and *h/d* (correlation > 0.8).

Table 2 Correlation among mean tree and stand variables for *R. pseudoacacia* L.: *BMD* = dry biomass, *RCD* = root collar diameter, *DBH* = diameter at breast height, *H* = height, *CB* = crown base, *Age* = tree age, *h_d* = height-diameter association, *SDI* = stand density index, *Bon* = classification, *NoP* = number of plants, *Prec* = Precipitation, *Temp* = temperature, *Sun* = sun duration, *DMI* = De Martonne Index, and *CWB* = climatic water balance (CARL ET AL., 2017a).

	BMD	RCD	DBH	H	CB	Age	h_d	SDI	Bon	NoP	Prec	Temp	Sun	DMI	CWB
BMD	1	0.95	0.95	0.87	0.72	0.89	-0.82	0.32	-0.19	-0.79	0.03	0.43	-0.01	-0.18	0.28
RCD		1	0.99	0.92	0.82	0.91	-0.84	0.47	-0.07	-0.84	-0.04	0.47	0.08	-0.28	0.18
DBH			1	0.94	0.85	0.90	-0.82	0.49	-0.03	-0.84	-0.07	0.45	0.11	-0.30	0.15
H				1	0.91	0.75	-0.71	0.68	0.23	-0.80	-0.10	0.54	0.31	-0.37	0.10
CB					1	0.69	-0.59	0.75	0.22	-0.67	-0.30	0.45	0.32	-0.55	0.08
Age						1	-0.86	0.29	-0.39	-0.77	-0.06	0.30	-0.03	-0.21	0.11
h_d							1	-0.20	0.34	0.93	0.12	-0.19	0.10	0.23	-0.29
SDI								1	0.58	-0.29	-0.07	0.64	0.64	-0.38	0.02
Bon									1	0.08	0.09	0.48	0.59	-0.14	-0.18
NoP										1	0.29	-0.20	-0.08	0.42	-0.16
Prec											1	0.42	-0.12	0.90	-0.06
Temp												1	0.53	-0.02	0.11
Sun													1	-0.39	-0.46
DMI														1	-0.12
CWB															1

In Table 3, the top four allometric biomass equations as general models for black locust growing in short rotation systems in Germany are presented.

Table 3 Comparison among the top four candidate models, *RCD* = root collar diameter, *H* = height, *h/hg* = stand normalized height, *h/d* = slenderness, *SE* = standard error of the estimate, *p*-value, R^2 = conditional coefficient of determination, *AIC* = Akaike's Information Criterion, and variance (CARL ET AL., 2017a).

Model	Variable	Coefficient	Estimate	SE	p-value	R^2	AIC	Variance
M01	Intercept	a	-2.65499	0.0436	< 0.001	0.9353	1890	0.2061
	RCD	b	2.29325	0.0239	< 0.001			
M02	Intercept	a	-3.50576	0.0649	< 0.001	0.9532	1466	0.1572
	RCD	b	1.52190	0.0403	< 0.001			
	H	c	1.20956	0.0541	< 0.001			
M03	Intercept	a	-3.40775	0.0794	< 0.001	0.9492	1437	0.1567
	RCD	b	1.50926	0.0399	< 0.001			
	H	c	0.58536	0.1183	< 0.001			
	h/hg	d	0.63665	0.1063	< 0.001			
M04	Intercept	a	-3.69133	0.0930	< 0.001	0.9510	1419	0.1528
	RCD	b	1.94216	0.0961	< 0.001			
	H	c	0.27772	0.1340	0.0383			
	h/hg	d	0.53084	0.1056	< 0.001			
	h/d	e	0.26262	0.0535	< 0.001			



Among the variables, *RCD* showed the strongest correlation with *BMD* (Table 2 and M_{01}). Model M_{02} , which combines *RCD* and *H*, showed the best conditional coefficient of determination ($cR^2 = 0.953$) among all models tested. Furthermore, M_{04} was the minimum adequate model based on AIC and variance. That is, a combination of *RCD*, *H*, stand normalized height (h/hg), and slenderness (h/d) (Table 3). Moreover, different biomass equations are necessary to calculate trees growth on former agricultural and mining sites, as well as core versus stump growth, because significant differences among the various conditions were recognized. Figure 9 presents the nonlinear least square relationship and allometric relationship of the double-logarithmic representation between *RCD* (cm) and aboveground woody biomass [kg], *H* (m) and aboveground woody biomass [kg] separated by previous land utilization (agriculture and mining) and growth (core and stump). In previous agricultural areas, trees accumulate at the same *RCD* more aboveground woody biomass then in previous mining areas. At the same *H*, the accumulated biomass in mining areas is higher than that in agricultural areas. Moreover, stumps accumulated more biomass at the equal *RCD* and core at the identical *H*, by comparing the equations of core and stump.

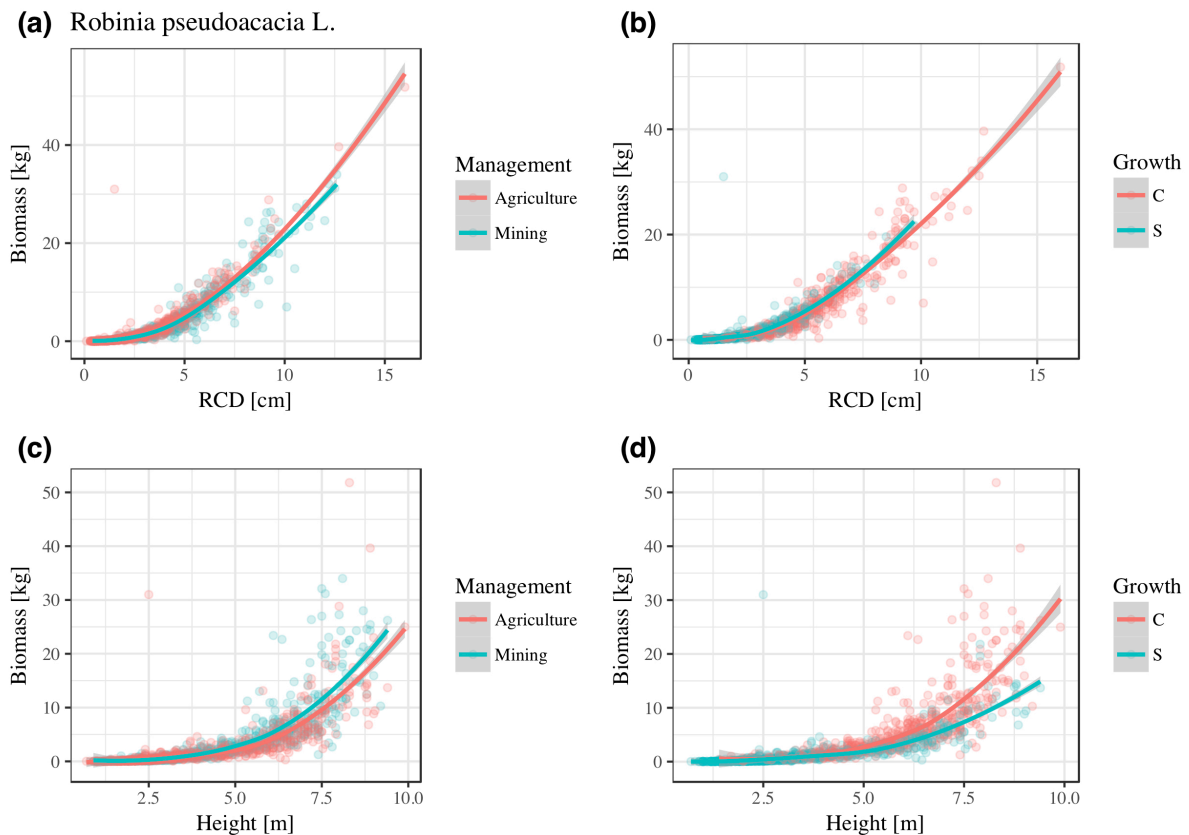


Figure 9 Allometric nonlinear least square relationship ((a),(b),(c),(d)) representation between *RCD* (cm) and aboveground woody biomass [kg] ((a),(b)) tree height (m) and aboveground woody biomass [kg] ((c),(d)) separated by previous land utilization (Agriculture and Mining) and growth (C = Core and S = Stump shoot) (CARL ET AL., 2017a).



The minimum and maximum absolute dry woody biomass productions of black locust were 1.0 and 13.8 t ha⁻¹ a⁻¹, respectively. Carbon sequestration per hectare and year ranged from 0.5 tons to 6.9 tons. The average carbon storage per hectare and year in the analyzed short rotation systems of black locust in Germany was 2.9 tons.

II.2.3.2 Competition

The supply or limitation of phosphorus and water as key belowground resources affected the slope and intercept of the size–growth relationship as well as the competition mode, by a varying growth partitioning significantly. The competition for belowground resources increased with decreasing P and AWC_r availability (–), while the competition for aboveground resources increased with increasing P and AWC_r availability (+). The results of the mixed-effects-model (Equation 3) are shown in Table 4. Estimates of the coefficients (b_1 , b_2 , b_3 , and b_4) showed that, with increasing values for BA , CI_{Light} , P , and AWC_r , the response variable gBA also increased. Furthermore, increasing values in the interactions of $CI_{Light} * P$ and $CI_{Light} * AWC_r$ ($-b_5$, $-b_6$) were associated with decreases in gBA values. Estimates of the coefficients $-b_5$ and $-b_6$ had the strongest effects on trees in fertile sites (high P and AWC_r values) with a high CI_{Light} value, primarily small trees with many light competitors. Consequently, the greatest slope (asymmetry) was observed in the most fertile sites because the growth partitioning of trees with many light competitors (i.e. small trees) exhibited the greatest reduction in the interaction among above- and belowground resources. Large trees in fertile sites benefitted from the greater influences of the estimates of the coefficients (b_1 , b_3 , and b_4) of BA , P , and AWC_r as well as the slight reduction as a result of the interactions of $CI_{Light} * P$ and $CI_{Light} * AWC_r$.

Table 4 Explanatory variables and the coefficient estimates of Equation 3. BA = basal area, CI_{Light} = sun-light competition index, P = Phosphorus (mg kg⁻¹), AWC_r = available water capacity of the roots, SE = standard error of the estimate, $StdDev$ = standard deviation, and $corr$ = correlation. Significance level: *** p <0.001, ** p <0.01, * p <0.05, ns not significant (CARL ET AL., 2018).

Variable	Coefficient	Estimate	SE	p-value	Variance	StdDev	Corr
	b_0	-4.7138	1.1272	<0.0001	0.74594	0.86368	
ln(BA)	b_1	0.9933	0.0961	<0.0001	0.06507	0.25510	-0.655
CI_{Light}	b_2	0.0028	0.0015	0.0616			
P	b_3	0.1074	0.0410	0.0397			
AWC_r	b_4	0.0122	0.0063	0.1007			
$CI_{Light} * P$	b_5	-0.0001	0.0001	0.0461			
$CI_{Light} * AWC_r$	b_6	-0.00002	< 0.0001	0.0306			

In contrast, on sites with increasing limitation of P and AWC_r , the influence of the explanatory variables P and AWC_r were much slighter than on fertile sites, and the slopes of the size–growth relationship was more symmetric and flattened (Figure 10). Additionally, the estimates remained significant (p <0.05) when including the random intercept and slope. Thus, the results were independent of random and unknown site-specific effects.



In Figure 10, the size–growth relationship results from Table 4 were plotted with defined varying values of the explanatory variables (+ ~ –). High P (+) and AWC_r (+) values showed that taller trees benefit and have disproportionately higher growth partitioning than smaller trees. As a result, in the BA – gBA relationship, the long dashed line was convex size asymmetric. Average P (~) and AWC_r (~) values resulted in convex size symmetric competition, whereas average P (~) and low AWC_r (–) values resulted in size symmetric competition. Hence, the competitiveness of trees increases proportionately with the tree size. Low P (–) and AWC_r (–) values displayed concave size symmetry. Additionally, taller trees received minor growth partitioning compared with stands of high P and AWC_r values.

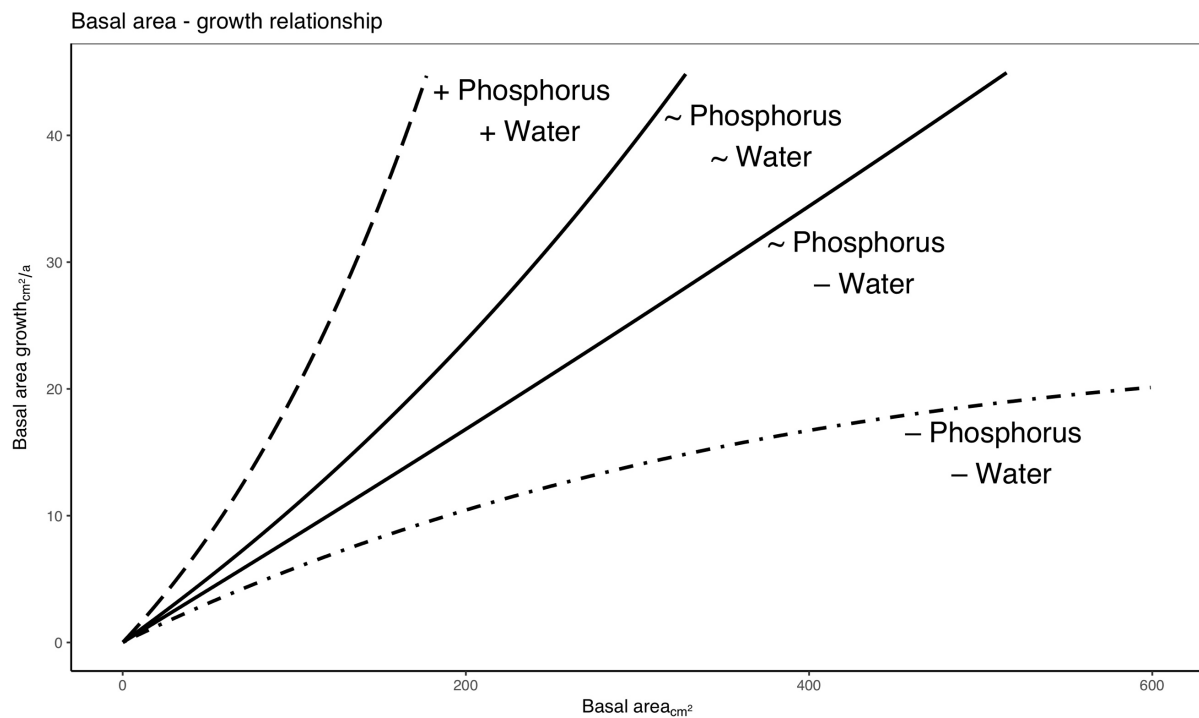


Figure 10 Basal area–basal area growth relationship. The long dashed line represents high phosphorus and water availability (+) and is convex size asymmetric, whereas the solid line with average phosphorus and water availability (~) is convex size symmetric. The solid line with average phosphorus values (~) and a low water availability (–) is size symmetric, while the dot-dash line with low phosphorus and water availability (–) is concave size symmetric (CARL ET AL., 2018).



‘Watching the bees among the flowers is an old and happy occupation, not only for children but their elders.’

PATRICK GEDDES, *Chapters in Modern Botany*, 1893



‘R. pseudoacacia flower and *A. mellifera*’

REMME, 2009



II.3 Ancillary use: Flower and Honey Potential

II.3.1 Introduction

Most available research focuses on the woody biomass production of trees. However, trees also provide other ancillary benefits, such as honey production, from which humans have benefitted for thousands of years by cultivating honeybees (*Apis mellifera*). Besides their role in honey production, honeybees also pollinate trees. Flower production is essential for the survival of most plant species in the world because it relates directly to the number of seeds produced. As a side effect, flowers provide ecosystem services, such as food for many insects via the flower–pollinator relationship.

Black locust starts flowering early, often at an age of six years (RÉDEI, 2013) or younger. The flowers of *R. pseudoacacia* are cream-white or pink with a pale yellow blotch at the center and measure 1–2.5 cm (ERTELD, 1952). The structure is typical for a papilionaceous flower with a pedicel, calix, one banner, two wings, two keel, nine fused stamens, and one free stamen (ROLOFF ET AL., 2010). The number of flowers per inflorescence (raceme) ranges from 10 to 25, and the inflorescence measures 10–25 cm long (DIRR, 1990). In Germany, the flowers open at the end of May or the beginning of June for four to six days, after the leaves have been developed (ROLOFF ET AL., 2010). The flowers are pollinated by insects, primarily by *Hymenopteran* insects, such as *A. mellifera*. In summer, a hive counts 30–50,000 honeybees (DACHMARKE, 2017), and in winter, 10–15,000 honeybees (IMKERVEREIN BÜCHERTAL, 2017). One flower produces 1.7–3.7 mg nectar in 24 hours during flower time (DROEGE, 1989). The sugar concentrations of the nectar is 34 to 67% Brix and for honey 80% Brix (DROEGE, 1989). *R. pseudoacacia* honey is light yellow and mild in flavor. Furthermore, beekeeper appreciate *R. pseudoacacia* honey and it is a popular, premium-priced natural product in most markets. In Hungary researchers started to improve nectar production systematically by plant breeding (RÉDEI, 2013). Today, in Hungary *R. pseudoacacia* honey is an important economical product. Thereof, 50–60% of the total honey produced in Hungary is *R. pseudoacacia* honey (RÉDEI, 2013). The highest nectar and honey yields in a common plantation achieved by *R. pseudoacacia* in Hungary were 836 kg ha⁻¹ nectar and 418 kg ha⁻¹ honey in an age of 15 years (RÉDEI, 2013). Nevertheless, flower detection is difficult, especially of trees. However, UAS allows the monitoring of small, specific, and detailed vegetation structures, such as flowers. In addition, UAS features quick turnaround times and low operating costs. Therefore, UAS data may provide an important supplement to data collected from satellites, airplanes, and the field. In the third research article (CARL ET AL., 2017b) the research objectives were (1) to adapt methods to classify flowers in black locust plantations via UAS red–green–blue (RGB) aerial imagery; and (2) to evaluate the number of flowers and the nectar production as well as to assess the habitat for honeybees and the honey potential.



II.3.2 Material and Methods

The study site, Lauchhammer, Brandenburg, Germany, is shown in Section II.2.2.1. Study Sites. Data collected for the flower analysis were subdivided into vertical (13 flying altitudes), horizontal (50 x 50 m² area), and flower tree (seven sample trees in bloom) analysis (Figure 11) in an eight-year-old black locust plantation in May and June 2017. For the vertical and horizontal analyses, a hexacopter took RGB (red, green, blue) pictures. Flower tree data were manually collected from the field; here, 500 flowers were counted, photographed, and weighed.

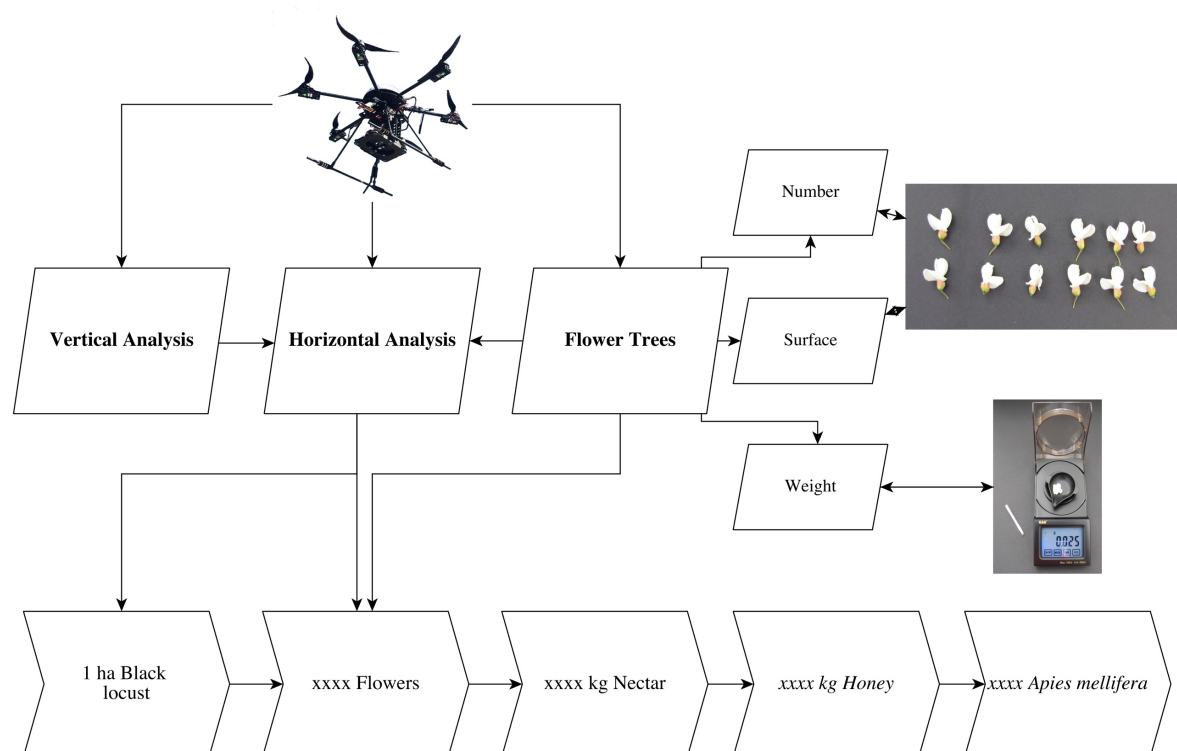


Figure 11 Flow chart showing analysis procedure: vertical, horizontal analysis, and flower trees. Example of surface flower analysis (2D) and the weighing process of the flowers for the volume analysis (3D) (CARL ET AL., 2017b).

ImageJ (SCHINDELIN ET AL., 2012; SCHNEIDER ET AL., 2012) was used for pixel-based image analysis. The blue channel was classified as the channel with the main differences between flowers and other vegetation (leaves, weeds, branches), which reflect poorly in the blue band. The white color of the flowers becomes strongly visible. The flower area was calculated after defining the threshold value for all three subdivisions of analysis: vertical (threshold 110–160), horizontal (threshold 140), and flower tree (threshold 140). Image analysis was evaluated with the confusion matrix presenting the overall accuracy and the kappa coefficient (GROUVEN ET AL., 2007). With the aid of the flower trees, the deviations among the UAS pictures and actual flower numbers could be estimated. To estimate nectar and honey production, as well as the number of honeybee's, literature data were used and two honey examples were analyzed in the laboratory. For statistical evaluation, R version 3.3.2 (R CORE TEAM, 2016) and the listed application packages (CARL ET AL., 2017b) were applied.



II.3.3 Results

Vertical analysis (Figure 12) showed that most flowers are detectable if the UAS flies as close as possible to the crowns (2.6 m above crowns). Estimates of flower surface area ranged from 2.97% to 0.03% as the flying altitude above the crowns increased from 2.6 m to 92.6 m.

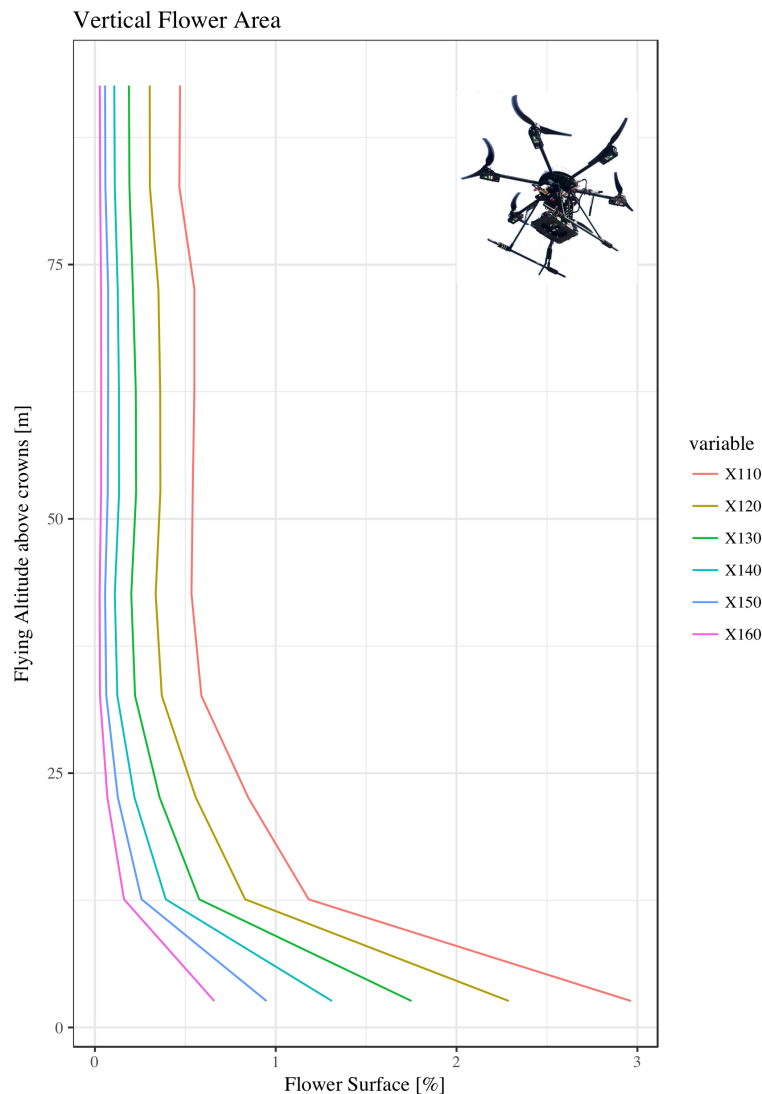


Figure 12 Vertical flower surface (%) for the flying altitudes 2.6 to 93.6 m above crowns. Graphs are based on different thresholds in the blue channel (CARL ET AL., 2017b).

Moreover, the best threshold for vertical, horizontal, and flower analyses was the threshold 140 in the blue channel with the highest overall accuracy (99.5%) and the highest kappa coefficient (0.913). The average detected flower area covered 1.33% of the total area under study (threshold 140), as estimated by the horizontal analysis. Flower tree analysis showed that, on average, the UAS detected 10.86% of the total 2D flower surface and 3.34% of the total 3D flower surface. The average flower surface was 1.92 cm^2 (2D), of which 0.21 cm^2 was detected by the UAS. In addition, the mean flower volume was 5.96 cm^3 (3D), of which 0.20 cm^3 was detected by the UAS.



Given the flower area determined through horizontal analysis and the detection rate of the analysis of flower trees, 5.3 million flowers per hectare were calculated. Overall, 5.3 million flowers may provide 87 kg of nectar and 70 kg of honey as well as, if just *R. pseudoacacia* bloom, may support a single bee hive for one year (Figure 13). Additionally, 19 different nectar-giving plants and 11 different nectarless plants were detected in the two black locust honey samples.

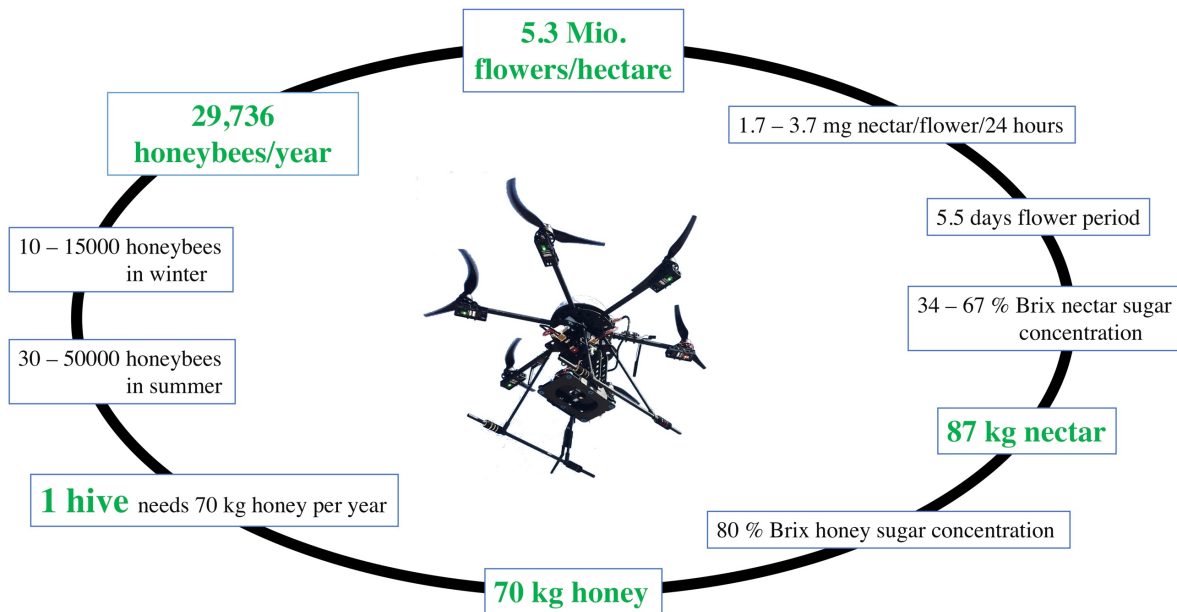


Figure 13 Flow chart connecting flowers, nectar, and honeybees per hectare. The green text data are calculated and the black text are data from literature (CARL ET AL., 2017b).

II.4 Risk – Reproduction and Invasiveness

II.4.1 Introduction

Beside positive effects, such as woody biomass production, reforestation of marginal land, and honey production, negative effects are not neglectable. One risk of black locust is its sprouting to neighboring areas, especially those in which endangered species are growing. The non-native species secretariat – NNSS (2018) defines invasiveness as the spread of non-native species in locations, where native ecosystem communities exist. Invasive species often change, damage, or destroy indigenous communities to the disadvantage of existing ecological structures and coexistence with humans. The EUROPEAN COMMISSION (2018) published Delivering Alien Invasive Species Inventories for Europe and noted that biological invasions by non-native or ‘alien’ species are one of the greatest threats to the ecological and economic well-being of the planet. Alien species can act as vectors for new diseases, alter ecosystem processes, change biodiversity, disrupt cultural landscapes, reduce the value of land and water for human activities and cause other socio-economic consequences for humans (EUROPEAN COMMISSION, 2018). In Europe, *R. pseudoacacia* has been declared as one of the most invasive non-native tree species and is listed as one of the 100 worst plant species (EUROPEAN COMMISSION, 2018).



Black locust reproduces generatively via seeds and vegetatively via stump shoots (Figure 14) and root suckers. Generative reproduction starts early, often at an age of six years (RÉDEI, 2013). The seed coat is hard and needs scarification before germination. In an average year, 120–150 kg ha⁻¹ of seeds are produced (RÉDEI, 2013). Further, it is possible to extract the seeds of the 5 cm layer of the litter resulting in 100 seeds m⁻¹ or 2,000–2,200 kg m⁻¹ in a 50-year-old stand (RÉDEI, 2013). Nevertheless, scarification intensity has not been researched much, particularly in seeds from an eight-year-old short rotation coppice. Thus, the average germination of black locust seeds after six different seed treatments was assessed by CARL ET AL. (2019).



Figure 14 Black locust seeds and a three-day-old black locust tree (left) and stump shoots (right).

A vegetative reproduction strategy is stump shoot sprouting after cutting (Figure 14). This trait is one of the reasons black locust is planted in short rotation systems. However, the sprouting intensity and dieback of black locust shoots with increasing shoot age in densely populated short rotation coppices has not yet been in the focus of research up to now. In the fourth research paper (CARL ET AL., 2019) the average survival of stump shoots was analyzed.

Furthermore, vegetative reproduction by layering (root suckers) is a strategy of spatial expansion. Nevertheless, there is not an effective method to determine the sprouting of tree species to neighboring areas. Similar to flower detection, there is a high spatial resolution necessary. Thereof, UAS is a key technology to detect the distribution of vegetation, particularly trees. In the research article of CARL ET AL. (2019), UAS were applied to observe black locust in the plantations as well as the distribution of black locust out of planting lines into neighboring areas. The research objectives were (1) to adapt methods to classify invasiveness of black locust via UAS-based RGB and NIR (near infrared) aerial imagery and derive 3D point clouds for estimation of the sprouting distance of black locust to the surrounding areas depending on their utilization; and (2) to test deep learning algorithms for the automatic classification of black locust under varying conditions in single images.



II.4.2 Material and Methods

Seeds were collected in winter 2016 from two eight-year-old *R. pseudoacacia* short rotation systems in Germany (Lauchhammer and Röblingen–Section II.2.2.1. Study Sites) and then stored in a seed bank (Thuringia, Germany). Seed analysis began in spring 2018 and ended in summer 2018. The seeds were seeded in sandy mineral soil in planting boxes (Figure 15). A total of 3,000 seeds were included: 100 seeds in five iterations for six treatments:

- I) seeds were seeded and watered,
- II) seeds were soaked for 24 h in water at a water temperature of 18 °C (64.4 °F) and then seeded and watered,
- III) seeds were stored at an air temperature of 45 °C (113 °F) for two hours, and thereafter stored at an air temperature of –20 °C (–4 °F) for a further two hours and then seeded and watered,
- IV) seeds were stored at an air temperature of 60 °C (140 °F) for two hours and thereafter stored for two hours at an air temperature of –20 °C (–4 °F) and then seeded and watered,
- V) seeds were scalded with hot water, seeded and watered, and
- VI) seeds were mechanically scarified, seeded and watered.

Stump shoot sample plots (Figure 15) in seven study sites were analyzed in winter 2016/2017. In total, 5,244 black locust stump shoots were investigated by measuring leafless stump shoots in 33 sample plots (Section II.2.2.1. Study Sites). Sample plot sizes ranged from 4–6 m in radius and contained a minimum of 150 stump shoots. The measured number of stump shoots per sample plot was divided by the number of established/planted black locust trees to calculate the average number of shoots per stump, per site, and per age class. ANOVA's were performed to evaluate the seed germination depending on seed treatment and shoots per stump depending on age class.

The UAS images (Figure 15) and field data for the spreading analysis were collected in 2018 in Damendorf (Section II.2.2.1. Study Sites). The site was planted in 2009 and harvested four years ago (2014). The analyzed total length was 2,124 m. Two UAS were used: Microdrones MD4–200 (MICRODRONES GMBH, 2017) and dji Mavic Pro (SZ DJI TECHNOLOGY CO. LTD., 2017). For georeferencing purposes, 13 ground control points were installed. RGB and NIR pictures were taken at flying altitudes of 15–100 m above ground. Two analytical methods were applied: (1) object-based image analysis (OBIA), and (2) a convolutional neural network (CNN). For OBIA, images were matched to create an orthomosaics and digital surface models (DSM) by using structure from motion. OBIA consists of two major steps: (A) segmentation and (B) classification. The three most important features are (a) the spectral information of the mean green value as black locust leaves have a specific light green value, (b) vegetation height (DSM - Digital Terrain Model), which can classify distinctions of vegetation height from grass (≤ 0.2) as well as trees in plantations (≤ 3 m) and forests (> 3 m), and the (c) neighbor related pixel values in contrast to neighboring pixel to capture black locust trees in the shadows. After classification, the detected areas were measured, and evaluation was performed with the aid of a confusion matrix.

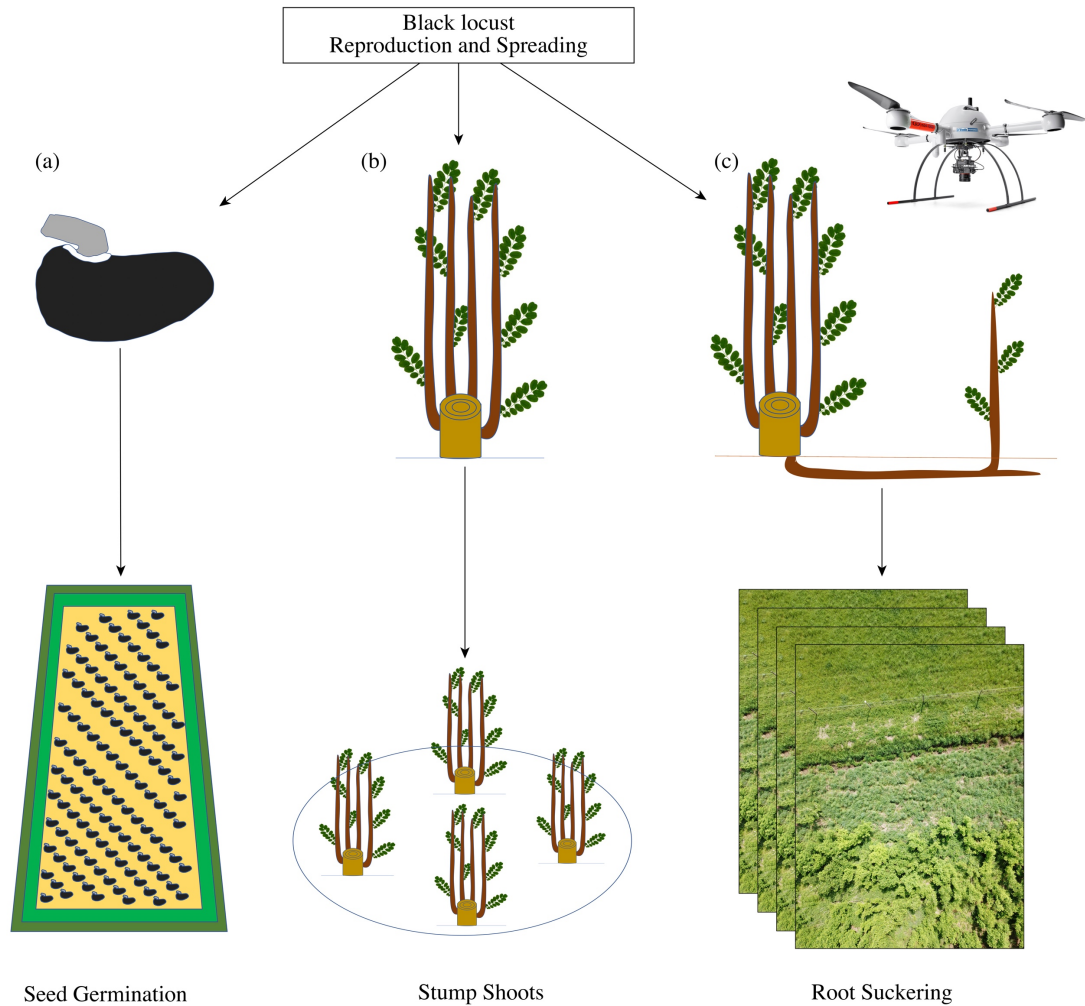


Figure 15 Flow chart showing the reproduction and spreading analysis: seed germination (a), stump shoot analysis (b), and root suckering (spreading) analysis (c). Seeds were seeded in planting boxes, and watered. Stump shoots were analyzed in sample plots. Spreading was estimated with the aid of UAS-images (CARL ET AL., 2019).

Deep learning algorithms were applied on single UAS images to classify black locust and non-black locust. Therefore, 1,000 RGB images were selected. Black locust was detected under varying light conditions (sunny and cloudy), flying altitudes (15 m, 30 m, and 100 m), UAS, and cameras; thus, different structures and colors. Different libraries (CARL ET AL., 2019) were applied in Python (PYTHON SOFTWARE FOUNDATION, 2018) via Jupyter Notebook (KLUYVER ET AL., 2016) to construct and calculate the CNN (SIMONYAN AND ZISSERMAN, 2014; NGUYEN AND ZEIGERMANN, 2018). The images were therefore split into training (80%) and validation (20%) data. The total number of trainable parameters is 4,787,330 per image. In sum, 3.83 billion training parameters were included, and the time required to fit the model was 24.2 min. To evaluate the applied CNNs, the loss and accuracy were computed.



II.4.3 Results

Seeds directly sown and watered achieved 6% germination. After soaking the seeds for 24 h, 8% of the seeds sprouted. After warming the seeds for two hours at 45°C/60°C and for two hours at –20 °C in warm-cold variation, germination reached 23%/69%. Scalding with hot water yielded a germination rate of 72% and mechanical scarification resulted in 90% of the seeds sprouting. Moreover, scalding of seeds achieved the highest standard error of the mean (Figure 16). The variance of all applied seed treatments differed significantly (p -value < 0.05).

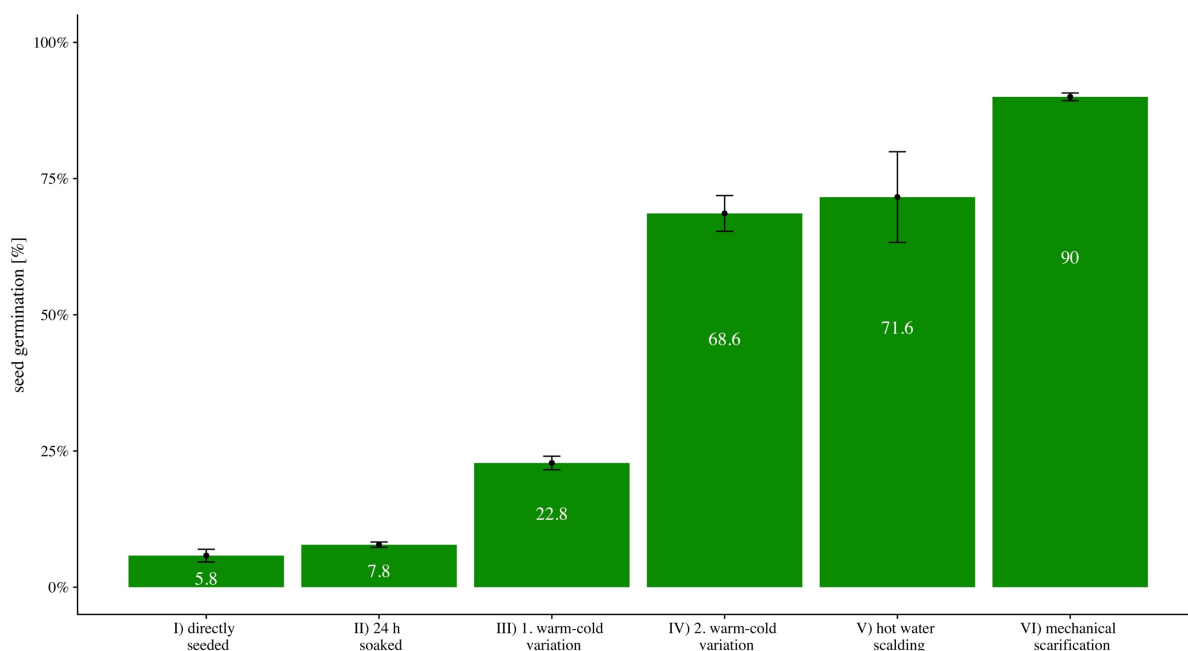


Figure 16 Black locust seed germination (%) by (I) directly sowing, (II) 24 h soaking, (III) warm-cold treatment for two hours at 45 °C and two hours at –20 °C, (IV) warm-cold treatment of two hours at 60 °C and two hours at –20 °C, (V) hot water scalding, and (VI) mechanical scarification. The error bars show the standard error of the mean per seed treatment. Database = 3000 seeds (CARL ET AL., 2019).

The average number of shoots per stump decreased with increasing age. In fact, the average number of shoots per stump was 4.2 in the first year after the last harvest, shoots aged one year. Two years after the last harvest, an average of 3.6 shoots per stump were found to be alive. In the third year after harvest, 2.2 shoots per stump continued to live. Furthermore, a statistically significant difference between age classes was determined by ANOVA.

In Figure 17, the proportion of black locust classified by OBIA in the five zones is shown as percentages depending on the surrounding area: meadow, farmland, dirt road, and pine forest. Furthermore, the average distance is presented with the aid of arrows. The farther away from the last planting line, the lower the proportion of black locust. The highest average distance, 2.26 m, is reached if the surrounding area is a dirt road (arrow in Figure 17). At 1.89 m, the second highest average distance is measured if the surrounding area is a meadow.



In the case of forest as the surrounding area, the average distance is 1.49 m. The smallest average distance, 1.16 m, is reached if the surrounding area is farmland. The overall accuracy of OBIA analysis is 97% and the overall kappa coefficient is 0.93.

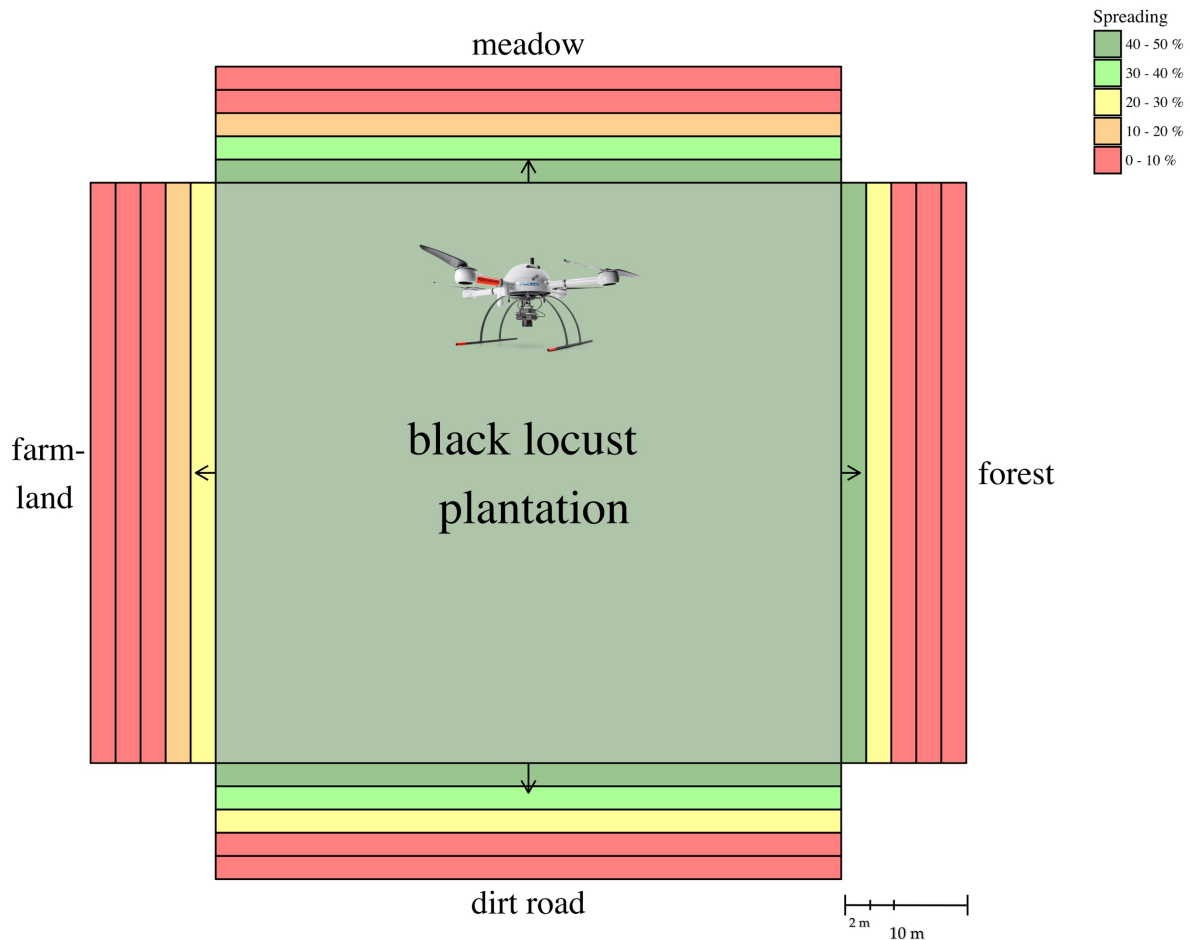
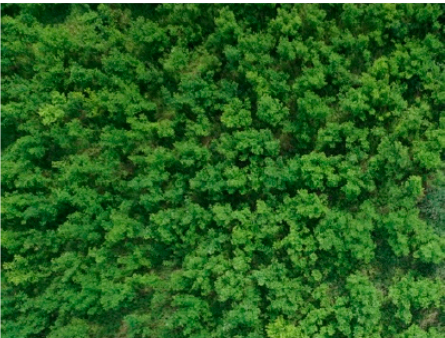
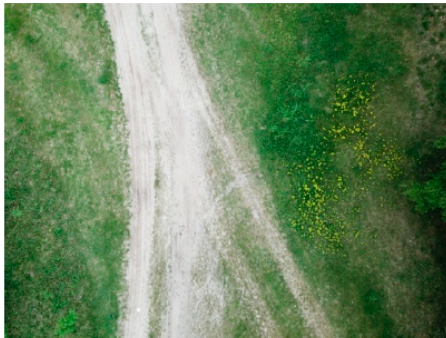
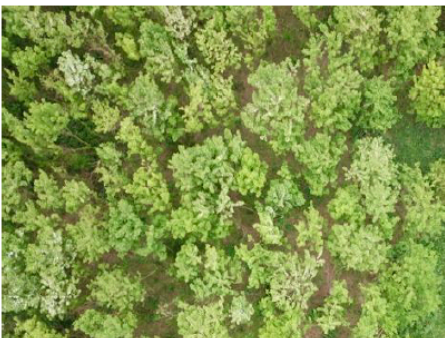
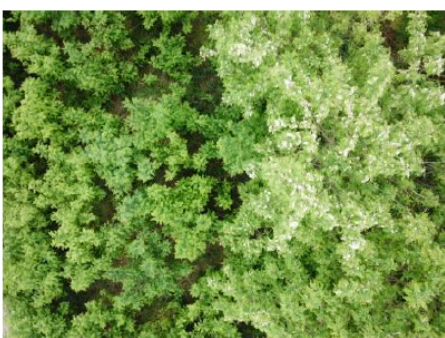
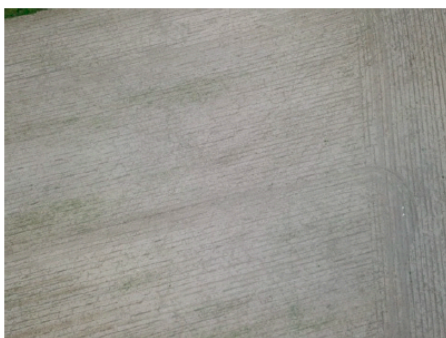


Figure 17 Spreading: outline average values of the areas; 0–2 m, 2–4 m, 4–6 m, 6–8 m, 8–10 m depending on the surrounding areas: meadow, farmland, dirt road, and pine forest. The spreading depends on the proportion of black locust; dark green stands for 40–50%, light green indicates 30–40%, yellow is 20–30%, orange 10–20%, and red 0–10%. The arrows show the average spreading distance of black locust: 2.26 m dirt road, 1.89 m meadow, 1.49 m forest, and 1.16 m farmland. Database = 2,124 m (CARL ET AL., 2019).

The results of the CNN model used to classify *R. pseudoacacia* and non-*R. pseudoacacia* is presented in Table 5. On the left-hand side are image examples of the test data of *R. pseudoacacia* (a) classifications with varying flying altitudes, light conditions, UAS, and cameras. On the right-hand side are examples of the test data of non-*R. pseudoacacia* (b) category. The CNN's accuracy in classifying *R. pseudoacacia* and non-*R. pseudoacacia* increased quickly. After a small number of iterations, the accuracy of the training and validation dataset increased by over 90%, ranging between 90 and 100% from iterations 5 to 50. The training accuracy reached 99.7%, and the test accuracy reached 99.5%. Finally, the training loss was 0.009, and the test loss was 0.027.



Table 5 Classification examples of the test data set; *R. pseudoacacia* (a) and non-*R. pseudoacacia* (b): meadow, dirt road, pine forest, and farmland. Pred is the abbreviation for Prediction. True and the green color of the labels show that the prediction and the class are equal (CARL ET AL., 2019).

(a) <i>Robinia pseudoacacia</i> L.	(b) Non <i>Robinia pseudoacacia</i> L.
	
1 True 1 Pred	0 True 0 Pred
	
1 True 1 Pred	0 True 0 Pred
	
1 True 1 Pred	0 True 0 Pred
	
1 True 1 Pred	0 True 0 Pred



II.5 Discussion

The evaluation of ecosystem services, potential and risk of the foreign tree species black locust is important to obtain a holistic perspective. However, the stand dynamics, honey potential, and spreading of black locust in short rotation coppices in Germany have not been analyzed until now. By applying biomass models, competition analysis, estimation of flower and honey potential, seed experiments, and spreading measurements, this thesis presents an overview of the potential and risk of black locust in short rotation coppices in a temperate climate zone. Black locust grows quickly, is able to fix nitrogen from air, reproduce generatively via flowers and seeds, as well as vegetatively via stump shoots and root suckers. These features improve the growth and survival of black locust in many ecosystems globally (AULD ET AL., 2003; FUENTES ET AL., 2010; CIERJACKS ET AL., 2013, LI AND LIU, 2014; AKATOV ET AL., 2016; VÍTKOVÁ ET AL., 2017). Thereby, the growth partitioning depends on the availability of phosphorus and water, and varies significantly between former mining and agricultural sites, as well as between harvested and non-harvested stands. Furthermore, a high flower amount results in a high food basis for animals, especially honeybees. The reproduction and spreading increases with greater damage to the seed coat and the harvest of black locust trees. Further favorable conditions are sandy soils and a high light availability.

An understanding of forest and plantation stand dynamics cannot be gained merely by analyzing organ or individual tree growth, as processes such as self-thinning or adaptation to density determine stand dynamics only at the stand level (PRETZSCH, 2009). Thereof, an understanding of stand or tree dynamics requires at least measurements from different spatial scales, e.g. tree and stand (PRETZSCH, 2009). Black locust is a light demanding tree species that is adapted to dryness and grows quickly. The present thesis shows that biomass production primarily depended on the diameter and height of black locust trees and stump shoots. This finding is in line with BÖHM ET AL. (2011), KRASZKIEWICZ (2013), STOLARSKI ET AL. (2015), and STANKOVA ET AL. (2016). The applied climate variables did not improve the allometric equations. However, by comparing model M_{01} , which includes the relationship between biomass and diameter, with the existing biomass equations (ELLIOTT ET AL., 2002; ENGEL ET AL., 2015; ANNIGHÖFER ET AL., 2016; VAN NEVEL ET AL., 2011; BÖHM ET AL., 2011; ANNIGHÖFER ET AL., 2012; BLUJDEA ET AL., 2012; LI AND LIU, 2014) of black locust, the slope of Germany_SRC – short rotation coppice was found to be the smallest. One reason could be varying final wood moisture levels. Nevertheless, in all studies the wood was dried until a constant weight was achieved. Strikingly, the allometric equations of LI AND LIU (2014) and CARL ET AL. (2017a) were approximately similar. LI AND LIU (2014) analyzed black locust, which was growing in the Loess Plateau in China. They describe that the soil profile in the study area consists of 65% sand, 24% silt, and 11% clay (LI AND LIU, 2014). The stands analyzed in the current thesis are also sandy soils with a clay content of 4.3–16.7%. The soil composition and, consequently, the available water and nutrients could explain the similarity between the studied black locust in Germany and in China.



FORRESTER ET AL. (2017) collected and merged biomass data and allometric equations of black locust (Figure 18, Mix). In the mix equation of FORRESTER ET AL. (2017), the data of the following studies are included: ANNIGHÖFER ET AL. (2012), BLUJDEA ET AL. (2012), ELLIOTT ET AL. (2002), LI AND LIU (2014), and VAN NEVEL ET AL. (2011). Such a combination is one approach to create an universal allometric equation for black locust. Nevertheless, there are high differences between the biomass equations on the tree level (Figure 18) and further on the stand level as, in this thesis, the biomass productivity ranged between 1.0–13.8 t ha⁻¹a⁻¹ in the analyzed short rotation systems.

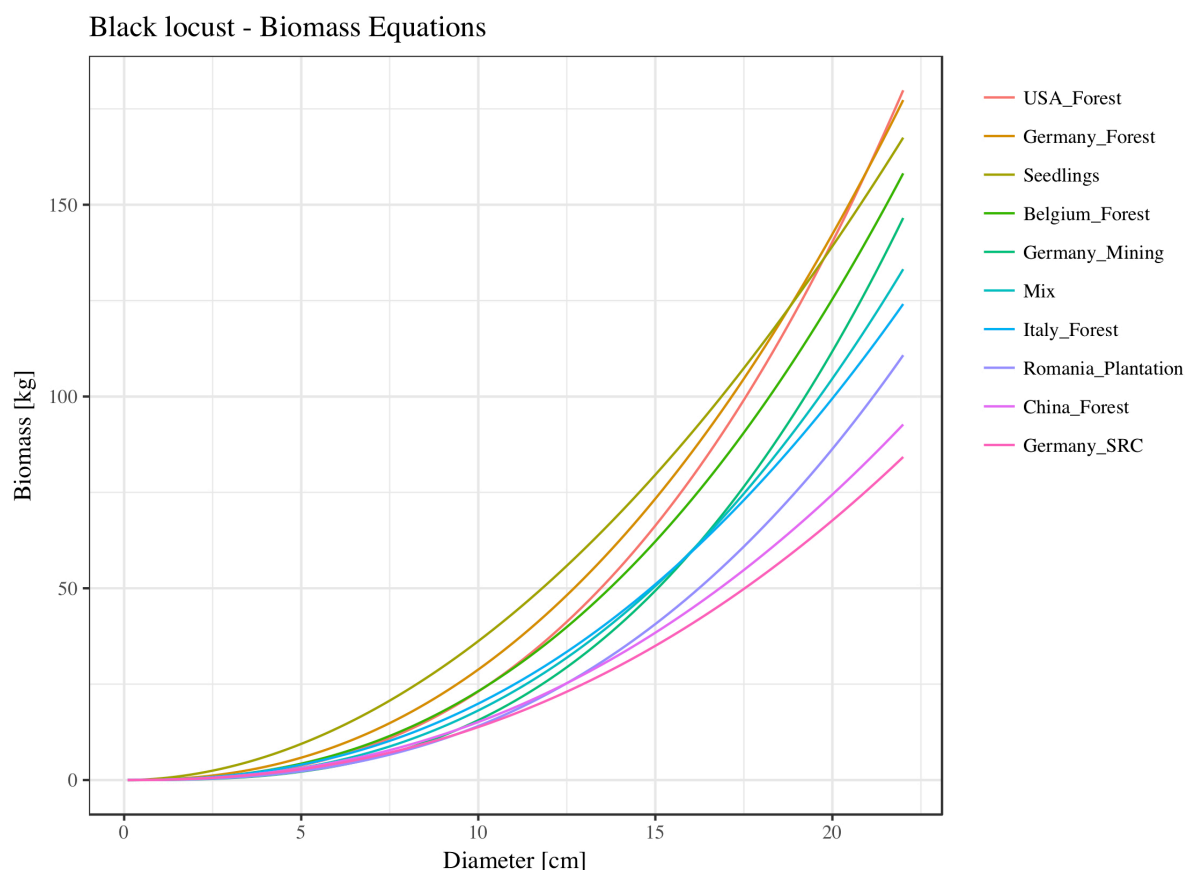


Figure 18 Biomass Equations of black locust published globally. This overview is not exhaustive: USA_Forest = ELLIOTT ET AL., 2002; Germany_Forest = ENGEL ET AL., 2015; Seedlings = ANNIGHÖFER ET AL., 2016; Belgium_Forest = VAN NEVEL ET AL., 2011; Germany_Mining = BÖHM ET AL., 2011; Mix = FORRESTER ET AL., 2017; Italy_Forest = ANNIGHÖFER ET AL., 2012; Romania_Plantation = BLUJDEA ET AL., 2012; China_Forest = LI AND LIU, 2014; Germany_SRC = CARL ET AL., 2017a.

Moreover, the biomass analysis presents that, depending on the former utilization (mining vs agricultural use) and growth type (harvested vs non-harvested), significant differences in the tree individual biomass and stand biomass production appear. One reason might be that the self-thinning and adaptation to density occur fairly quickly in harvested short rotation stands. Shoots per stump decreased from 4.2 in the first year after the last harvest to 3.6 shoots per stump in the second year after the last harvest to 2.2 shoots per stump in the third year after harvest. This findings corresponds to previous observations that black locust mortality increases in plantations with high planting density (CLARK, 1954; GEYER, 1989).



The intensity of dieback and significant growth differences among black locust trees in former mining and agricultural sites may also depend on the above- and belowground resources. As belowground resources, water and phosphorus are key drivers of competition among black locust trees and stump shoots. Accordingly, by increasing water and phosphorus availability, the competition for aboveground resources, primarily sunlight among black locust trees and stump shoots, increases. At this point it is important to note, that compared with other plant families, members of the *Fabaceae* require more phosphorus for their root development, and energy-driven processes that are central to maintaining of their mutualistic relationship with nitrogen-fixing rhizobia (OLIVERA ET AL., 2004; HERNANDEZ ET AL., 2009; ROTARU AND SINCLAIR, 2009; CABEZA ET AL., 2014). Moreover, *Fabaceae* like black locust are able to compensate for limited available soil nitrogen. Therefore, it seems that plant-available phosphorus appears to improve the phosphorus and nitrogen cycles of the *Fabaceae* appreciably. This effect plays a key role in the growth partitioning, competition mode, and stand dynamics of *R. pseudoacacia*.

For forest ecosystem management and forest practice it is important that the competition as well as the optimal tree density for high biomass production differed according to the water and phosphorus availability. In sites with high water and phosphorus availability (rich sites), lower stand densities and shorter harvesting intervals may be possible as self-thinning of the understory trees and stump shoots occurs quickly. In addition, in rich sites, the wood biomass (quality) increases, but the trees and shoots per stump (quantity) decreases. This feature could be also interpreted as a risk-minimizing and quality-improving aspect. The slenderness per tree and stump shoot is reduced, and the risk of breaking due to wind or weight, e.g. abundant seeds, is decreased. The wood quality of the surviving trees and shoots increases as the relative bark proportion decreases and the relative wood proportion per shoot increases. Furthermore, black locust wood differs among juvenile and adult trees (DÜNISCH ET AL., 2007; KOCH AND DÜNISCH, 2008; DÜNISCH ET AL., 2010; LATORRACA ET AL., 2011). Hence, as the black locust tree or shoot becomes older, its wood quality could be improved for energy use and timber construction.

Furthermore, to improve the life cycle (WOLF, 2017) of the black locust wood and to the use of wood in cascades (HÖGLMEIER ET AL., 2013, 2015), instead of energy usage, the timber could be processed alternatively as saw log, playground devices, working tool, pile (wine), underground mining timber, and veneer (ROLOFF ET AL., 2010; FINDEISEN, 2017; DITTRICH, 2017). Nevertheless, a problem of old black locust trees might be the core rot, as described by FINDEISEN (2017). Future studies could focus on the best planting distances for black locust to improve the target biomass and wood production. Moreover, as the effects of the phosphorus flux are not clearly understood (BOL ET AL., 2016; LANG ET AL., 2016), the influence of phosphorus on the processes of growth partitioning to wood, leaf, or cell could be investigated in future work. To improve the current knowledge, correlations among growth and sap flow (STEPPE ET AL., 2015, 2016; TURCSÁN ET AL., 2016), as well as detailed soil data, and genetic relationships could be include in newer models.



Detailed structural stand analysis could be accomplished via terrestrial laser scanning (TLS) methodologies (BAYER, 2018), LiDAR – light detection and ranging (ABDULLAHI, 2018), panoramic (360°) images of the forest stands, and UAS pictures to generate 3D point clouds (TOMAŠTÍK ET AL., 2017; NEVALAINEN ET AL., 2017).

Investigation of stand dynamics is important to estimate the biomass production and carbon storage of trees. Nevertheless, tree species, particularly black locust, offer other ecosystem services, such as flower production, which serves as a food basis for many insects and an estimate for honey production. Based on the UAS and field-collected data, 5.3 million flowers were estimated to be produced by an eight-year-old one-hectare black locust plantation in Lauchhammer for 2017. This flower amount produces an average of 87 kg of nectar. The honey, with 80% Brix sugar concentration, are 70 kg or 140 honey jars (500 g). One honeybee hive could survive for one year with 70 kg of honey as a food basis (DACHMARKE, 2017). Nevertheless, honey analysis in the laboratory showed that the honey of one bee hive includes the nectar and pollen of 30 different plant species. These results indicate that, during the flowering time of *R. pseudoacacia*, many other plant species produced energy spending nectar and pollen for honeybees. The focus of this study has been on a monoculture (plantation). However, biodiversity and ecosystem health benefit from the presence of many different plant species. Thus, the analysis of flowers is important to understand ecosystems worldwide. This approach provides natural and forest scientists, politicians, and laymen new insights into complex ecosystems, such as forests.

In this thesis, UAS technology was applied for the analysis of flowers and spreading. Whereas, biomass and flower production are primarily evaluated as positive aspects and form part of the potential of black locust, is the consideration of the reproduction and spreading part of silvicultural and ecological discussions as negative aspect of black locust. Hence, analysis of the seed germination and invasiveness of black locust in short rotation systems is described in the current thesis. Black locust seeds have a hard and impermeable seed coat (VINES, 1960; SCHUBERT, 1998), and seed germination increases with increasing warm–cold variation, hot water scalding, and mechanical scarification. This is in line with VINES (1960) and RÉDEI ET AL. (2012). Cracking and opening of the seed coat of black locust is necessary for successful germination. As black locust produces a large number of seeds (VÍTKOVÁ ET AL., 2017), the reproductive potential of this species via seeds is high. Nevertheless, the spreading of black locust short rotation coppices appears to take place primarily via root suckering (DOOLEY, 2003). A maximum average sprouting distance of 2.3 m is achieved if the surrounding area is a dirt road. Black locust root sprouting achieved 1.9 m towards the direction of a meadow, 1.5 m towards a forest, and 1.2 m towards a field. Moreover, with increasing distance, the proportion of black locust root suckering decreases, as shown in the analyzed zones. This finding is in line with CROSTI ET AL. (2016), who described that abandoned agricultural land is highly prone to black locust colonization. The spread was minimal in a zone with orchards and in semi-natural woodland (CROSTI ET AL., 2016). Thus, the distribution is high in areas with a high light availability.



Further, the proportion decreases the closer to woodland or forest. The lesser distribution if there is farmland could be the tillage by the land owner and the destroying of the growing root suckering of black locust. To validate the present results, black locust stands with further neighboring ecosystems could be investigated.

Moreover, there are many more applications for UAS in forest science, forestry, biology, ecology, landscape management and so on. The possibility to produce 3D point clouds by a high overlapping of the images is a database to analyze different height and stand structures. Further vegetation, flower, and tree indices show the possibility to detect individual species and vegetation elements. The applicability of UAS provides a new datasets to study stands and environmental conditions. Furthermore, in this thesis, most of the software used is open-source; this detail is important in facilitating repetition of the described methodologies in future studies without the high cost associated with software technologies. Open source software, such as R (R CORE TEAM, 2016), ImageJ (SCHINDELIN ET AL., 2012; SCHNEIDER ET AL., 2012), QGIS (QGIS DEVELOPMENT TEAM, 2018), and Python (PYTHON SOFTWARE FOUNDATION, 2018), are powerful and may be applied to various fields via packages, plugins, libraries for e.g. numerical and categorical data, images, 3D point cloud analysis, and statistics. Moreover, mixed-effects models were applied in this research. Mixed-effects models have the advantage that fixed and random effects are included. Thereby, fixed effects present the population effects (overall components) and random effects show the possible influence of sites or subject-specific unknown effects (ZUUR ET AL., 2009), which could occur if more than one measurement was done at an individual tree, site, area, etc. It is an approach for complex data analysis and modelling. Furthermore, deep learning, especially convolutional neural networks, for image classification are learning systems that enables the possibility to detect objects under varying conditions, similar to the way human eyes accomplish the same task. Classification of black locust in single images under varying conditions was successfully modeled, thus illustrating the potential of deep learning algorithms.

For local management, general biomass equations can be used to calculate the current biomass, proceeds, profit, and, consequently, the optimal harvesting time. To improve the calculated results, specific models for former agricultural and mining areas, as well as growth by core or stump, are available. Furthermore, in rich sites (high phosphorus and water availability), harvesting is possible earlier than on poor sites to foster individual trees and to benefit economically. The method and the results of the flower and spreading analyses provide the applicability of UAS. Beekeepers and natural scientists, in general, can benefit from these analyzes. For the future, there is the possibility to improve the biodiversity analysis and, furthermore, it could be possible to model the number of flowers per tree influenced for example by yield data and weather (climate). Moreover, there is a research gap in the behavior of black locust in mixed stands with native or non-native species. Future studies could focus on this aspect to classify interspecific competition.



This thesis has not the claim to give a complete overview of the features of black locust. However, stand dynamics, biomass production, competition, biodiversity, honey potential, and invasiveness as a risk of black locust in short rotation systems were analyzed, and methods were developed for further analysis of black locust and other tree species. Black locust, especially the sprouting of root suckers, stump shoots, and seeds should be further investigated. In areas where the planting of other tree species is difficult, such as in marginal areas or former open-cast mining areas, black locust is an alternative tree species that can prevent erosion of embankments, revegetate landscape, and accumulate soil with nitrogen. Thereof, it might be more sustainable (VON CARLOWITZ, 1713) to plant a non-native species like black locust, then to have wasteland. However, it depends on the purposes of the land owner or manager, which tree species are planted and promoted. Native species and mixed forest stands have the advantage to reduce ecological and economical risk (DEL RÍO ET AL., 2017, BAUHUS ET AL., 2017). Furthermore, mixed stands increase stand productivity (PRETZSCH ET AL., 2015). Nevertheless, short rotation systems are an approach to produce renewable energy with fast growing tree species. For wasteland and dry agricultural areas, short rotation coppice could be an alternative land utilization, but it should be not in conflict with food production, biodiversity loss, as well as further ecological and sociological aspects. To sum up, black locust has grown in Europe over the past 417 years since being introduced to the continent in 1601 by JEAN ROBIN (VADAS, 1914), and it appears in many European countries. Positive and negative aspects of the tree species were analyzed and discussed in former studies and in the current thesis.

II.6 Conclusion

This thesis provides an overview of the stand dynamics, honey potential, and invasiveness of black locust in short rotation systems in Germany. The stand dynamics focused on the biomass production, carbon storage, and growth partitioning among black locust trees and stump shoots. Therefore, with the aid of a Pearson correlation matrix, the main variables were selected and four allometric biomass models were calculated and presented. Furthermore, the biomass equations differ among former agricultural and mining areas and among harvested and non-harvested stands. The minimum and maximum absolute dry woody biomass production per stand were found to be 1.0 and 13.8 t ha⁻¹a⁻¹, respectively. Carbon sequestration per hectare and year ranged from 0.5 tons to 6.9 tons. The key drivers of the competition mode of *R. pseudoacacia*, by their effect on growth partitioning, were phosphorus and water, which was estimated by applying the size-growth relationship in a mixed-effects model. With increasing supply of phosphorus and water, competition for sunlight increased and large trees grew disproportionately more than smaller trees. Moreover, a total 5.3 million flowers that are able to produce 87 kg of nectar (70 kg of honey) were produced by an eight-years-old black locust short rotation coppice in the year 2017, which is the food basis for one bee hive for one year. Additionally, there might be the possibility for beekeepers to produce 140 honey jars (500 g).



Therefore, the flower analysis was split into vertical, horizontal (UAS technology), and flower tree analyses (terrestrial manual analysis). The applied seed experiments showed that seed germination increases with increasing warm–cold variation, hot water scalding, and mechanical scarification of the hard and impermeable seed coat. Moreover, the average sprouting distance was measured with the aid of UAS images and found to be highest if the neighboring area is a dirt road and a meadow. Less spreading is achieved if the neighboring area is a forest or farmland. This thesis provides results of the potential and risk of black locust in short rotation coppice for forest managers, natural and ecological scientists, politicians, and laymen.

II.7 Prospects

To achieve the adequate evaluation and management of black locust in short rotation systems, knowledge of its potential and risk is crucial. Therefore, this thesis describes the stand dynamics, honey potential, and invasiveness of black locust in short rotation systems. However, to attain an improved holistic perspective of black locust, it is important to focus on further non-investigated aspects, such as the appearance, biology, ecology, silvicultural treatment, wood quality, and timber processing (Figure 1). Nevertheless, the in this thesis analyzed topics could be also further investigated. Thereby, the high variability of the biomass and growth partitioning at the tree and stand levels imposes further challenges on future research: what controls this variability, besides water and phosphorus (e.g. biological, physical, and/or chemical processes), and to which extent can it be maintained by treatment and management (e.g. planting distances and harvesting periods). A possible way to tackle these issues is to combine stand, tree, and cell analyses to gain insights into the different hierarchical scales and combine them. Moreover, indications that the growth and biomass yield of black locust are improved by mixing with tree species have been observed (OLIVEIRA ET AL., 2018). Hence, a better understanding of inter-specific competition could improve the carbon sequestration and biomass potential of black locust in mixed stands and is therefore a next step of research. A more integrative approach is needed to incorporate also the stand parameters and biomass with wood quality and timber processing, similar to that described by RAIS (2015). As the number of data and influencing factors mostly increase, the use of robust and comprehensive models becomes increasingly important. A promising alternative and supplementary approach to mixed-effects models can be found in generalized additive models (GAM), as applied by ZELLER ET AL. (2018).

Flower and seed production of tree species varies over time and space, comparable to tree growth, which is mainly analyzed via tree-ring measurements or long-term permanent plots. In a wider sense, the mind-set of tree ring analysis could be also be applied to flower and seed analyses over time and space, as well as to pure and mixed stands. A better understanding of flower production, interactions of the climate, soil, and stand growth with the number of flowers, as well as the flower–insect relationships may, therefore, contribute to a more ecological perspective of flower interactions.



Better knowledge of seed germination in natural environments, for example, after a fire event or under extreme weather conditions, such as dry periods, heavy rain, or wind, require seed germination experiments in natural but observable conditions.

Short rotation systems are a specific management practice with many specifications and features different from those of high forest stands. Therefore, the results presented in this thesis can be regarded as a rough overview of the attributes of black locust in North-East Germany. Expansion of data analysis to the European and global scales is a next step and could help facilitate evaluations on ecological gradients.

UAS integration into forest inventory and interdisciplinary ecological research provides standard and novel forest attributes. However, further research on spreading analyses and forest attributes is required for multilayered stands and stands with a closed canopy. A promising approach for that are laser technologies, such as TLS or LiDAR, which were successfully applied to forest stands by BAYER (2018) and ABDULLAHI (2018), respectively. A further possible future technology are UAS flying in a forest stands equipped with different camera systems and laser technologies. Thereby, TLS (BAYER, 2018), LiDAR (ABDULLAHI, 2018), as well as UAS over and in forest stands provide possibilities for a comprehensive data collection to generate a virtual reality of forest stands and trees. Thereby, the questions: what are the spreading distances per year of black locust to and in further ecosystem structures and how is the spreading of black locust changing with increasing average temperatures and low precipitation, could be also focused.

A promising approach to generate information from the measured data (1D), images (2D), and 3D point clouds (3D) is machine learning, such as deep learning methods, including CNN, which was successfully applied to classify black locust in single images in this dissertation. As machine learning algorithms are applicable to 1D, 2D, and 3D data, they can be mixed further. A framework for combining numerical and categorical data (1D) with image data (2D) in a machine learning model was demonstrated by ROSEBROCK (2019). Multiple inputs and mixed data are a promising perspective to combine different forest parameters and dimensions (1–3D). Furthermore, automatic tree species detection may improve and accelerate forest inventories and invasiveness analyses. Therefore, the training and testing of object (e.g. tree, forest, plantation) detection with the aid of deep learning is a further and important next step in future research.



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List of Abbreviations

1D	One- Dimensional
2D	Two-Dimensional
3D	Three-Dimensional
AIC	Akaike Information Criterion
ANOVA	Analysis of Variance
AWC _r	Available Water Capacity for the Roots
BA	Basal Area
BM	Biomass (dry)
C	Core growth
CB	Crown base
CI _{Light}	Sunlight Competition Index
CNN	Convolutional Neural Network
Cp	Competition Analysis
CVH	Chinese Virtual Herbarium
CWB	Climatic Water Balance
d	Diameter
DBH	Diameter at Breast Height
DIN	Deutsches Institut für Normung
DMI	De Martonne Index
DSM	Digital Surface Model
DWD	Deutscher Wetterdienst
F	Flower Analysis
FHE	Fachhochschule Erfurt
GBIF	Global Biodiversity Information Facility
H	Height
h/d	Slenderness
hg	Average Stand Height
I	Invasiveness Analysis
IPCC	Intergovernmental Panel on Climate Change
L	LINNE
LiDAR	Light Detection and Ranging
mya	million years ago
NIR	Near infrared
NNSS	Non-Native Species Secretariat
NoP	Number of plants
OBIA	Object-Based Image Analysis
P	Phosphorus
„R ²	Conditional Coefficient of Determination
RCD	Root collar diameter
RGB	Red-Green-Blue
S	Stump growth
Sc	Seed Collection
SDI	Stand Density Index
SE	Standard Error
Spec	Species
SRC	Short Rotation Coppice
TLS	Terrestrial Laser Scanner
UAV	Unmanned Aerial Vehicles
UAS	Unmanned Aerial System



List of Species

<i>Apis mellifera</i>	Honeybee	Westliche Honigbiene
<i>Aesculus hippocastanum</i>	Horse-chestnut	Gewöhnliche Rosskastanie
<i>Asteraceae</i>	Daisy family	Korbblütler
<i>Betula ssp.</i>	Birch	Birkengewächse
<i>Brassicaceae</i>	Cruciferous	Kreuzblütler
<i>Cornus</i>	Dogwood	Hartriegel
<i>Cyanus segetum</i>	Cornflower	Kornblume
<i>Eucalyptus nitens</i>	Shining gum	Glanz-Eukalyptus
<i>Fabaceae</i>	Legume	Hülsenfrüchtler, Leguminose
<i>Filipendula ulmaria</i>	Bridewort	Echte Mädesüß
<i>Fragaria x ananassa</i>	Strawberry	Erdbeere
<i>Frangula alnus</i>	Buckthorn	Faulbaum
<i>Gleditsia</i>	Honey locust	Gleditschien
<i>Hymenoptera</i>	Hymenopteran	Hautflügler
<i>Liliaceae</i>	Lily family	Liliengewächse
<i>Narcissus spec.</i>	Daffodils	Narzissen
<i>Papaver</i>	Poppy	Mohn
<i>Pinus spec.</i>	Pine	Kiefern
<i>Poaceae</i>	Sweet grass	Süßgräser
<i>Populus spec.</i>	Poplar	Pappeln
<i>Pyrinae</i>	Maloideae	Kernobstgewächse
<i>Rhizobia spec.</i>	Rhizobia	Knöllchenbakterien
<i>Robinia pseudoacacia L.</i>	Black locust	Gewöhnliche Robinie
<i>Rosaceae</i>	Rose family	Rosengewächse
<i>Rubus spec.</i>	Rubus	Rosenartige
<i>Salix spec.</i>	Willow	Weide
<i>Sambucus spec.</i>	European elder	Holunder
<i>Sinapis</i>	Charlock	Senfe
<i>Tilia ssp.</i>	Lime, linden	Linden
<i>Trifolium</i>	Clover	Klee
<i>Weigela</i>	Weigela	Weigilien
<i>Quercus robur</i>	Pedunculated oak	Stieleiche



Appendix

A. Supplementary Material



Figure 19 Time scale development of the yield tables, growth and biomass equation of black locust. This overview is not complete. Identical colors indicate that the data were estimated from the same country.



B. Published Articles

Article 1: Biomass - Allometric Equation

Carl, C., Biber, P., Landgraf, D., Buras, A., Pretzsch, H. (2017a). Allometric Models to Predict Above-ground Woody Biomass of Black Locust (*Robinia pseudoacacia* L.) in Short Rotation Coppice in Previous Mining and Agricultural Areas in Germany. *Forests*, 8(9), 328, doi:10.3390/f8090328, <http://www.mdpi.com/1999-4907/8/9/328>.

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Carl et al. 2017a

Article

Allometric Models to Predict Aboveground Woody Biomass of Black Locust (*Robinia pseudoacacia* L.) in Short Rotation Coppice in Previous Mining and Agricultural Areas in Germany

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Abstract: Black locust is a drought-resistant tree species with high biomass productivity during juvenility; it is able to thrive on wastelands, such as former brown coal fields and dry agricultural areas. However, research conducted on this species in such areas is limited. This paper aims to provide a basis for predicting tree woody biomass for black locust based on tree, competition, and site variables at 14 sites in northeast Germany that were previously utilized for mining or agriculture. The study areas, which are located in an area covering 320 km × 280 km, are characterized by a variety of climatic and soil conditions. Influential variables, including tree parameters, competition, and climatic parameters were considered. Allometric biomass models were employed. The findings show that the most important parameters are tree and competition variables. Different former land utilizations, such as mining or agriculture, as well as growth by cores or stumps, significantly influenced aboveground woody biomass production. The new biomass models developed as part of this study can be applied to calculate woody biomass production and carbon sequestration of *Robinia pseudoacacia* L. in short rotation coppices in previous mining and agricultural areas.

Keywords: black locust; allometric models; short rotation coppice; mining; agriculture; carbon sequestration

1. Introduction

Robinia pseudoacacia L. was one of the first North American tree species to be introduced into Europe at the beginning of the 17th century [1,2]. It is also one of the most widely planted woody species in the world [3], and the third most important deciduous tree species, after *Populus* and *Eucalyptus*, for plantations, particularly short rotation coppices (SRCs). Fast growing trees are important worldwide. In Brazil, for example, *Eucalyptus* is crucial for pulp and paper production [4]. In Europe and particularly Germany, a change in energy policies towards reduction in use of fossil fuels and towards a stronger use of renewable energy, as outlined in the “Erneuerbare Energien Gesetz” (EEG 2004) [5], marked an increase in fast-growing tree production. Short rotation coppice can have a rotation cycle of 2–3 (short), 4–10 (medium), or 10–20 years (long), depending on management objectives [6,7]. To initiate SRCs, black locust seedlings are typically cultivated for one or two years

in tree nurseries and thereafter grown in plantations (in the following referred to as core growth). After harvesting (first, second, etc., rotation) secondary stocks resprout from the stump (referred to as stump growth). Black locust is not only a fast-growing tree species, but like many pioneer trees, it is an undemanding species. It grows in areas with soil pH up to 8 [8], and since it belongs to the legume family it is capable of nitrogen fixation (N) [9]. Therefore, black locust also survived in areas with less nutrients and limited water supply, such as previous open-cast mining areas [10]. Other extremely dry and sometimes salty environments where it survives are urban environments [11]. Black locust is a tree with many benefits. However, there are also those who express concerns about its invasiveness [12–14].

Studies and feedback by practitioners have shown that the yields of SRC, as well as forest biomass production, are affected by a number of interrelated factors, such as plant species provenance [3], age, planting distance, stand density [15–17], soil conditions [18,19], previous land use [20], harvest frequency, and climatic conditions [11,21,22]. In central Europe, the ecology of black locust has been studied for over 100 years [2,3,23–25]. Overall, growth and biomass productivity is one of its most important features, particularly for commercial forestry [26–30]. Research analyzing the growth of juvenile *Robinia pseudoacacia* L. in Europe has been conducted in Hungary [31,32], Austria [33], Italy [34], Bulgaria [35], Poland [19,36], and Germany [20,37–42]. In Germany, the development of allometric models has so far focused on forestry stands [37,41], seedlings [42], and in one case, on a post-mining area [20]. To date, there is no allometric equation available for the calculation of woody biomass in SRC in previous mining and agricultural areas.

To fill this research gap, the objectives of this study were (1) to develop allometric biomass equations for *Robinia pseudoacacia* L. growing in SRC in Germany; (2) to assess whether the inclusion of competition- and climate variables improves predictions from allometric biomass equations; (3) to determine if different biomass equations are necessary for trees growing on former agricultural and mining sites, as well as core versus stump growth; and (4) to determine the absolute woody biomass production and carbon storage per hectare and per year in the analyzed study stands (SRC) for black locust in Germany.

2. Materials and Methods

2.1. Site Description

Trees were collected in northeastern Germany, in the federal states of Brandenburg, Saxony-Anhalt, and Mecklenburg-Western Pomerania (Table 1, Figure 1). The investigated stands represent 2.4% of the area afforested with black locust in Germany [43].

The 14 study sites differ considerably in regards to their climate and soil characteristics. The annual precipitation ranges between 500 mm and 621 mm and mean annual temperature (1965–2015) ranges between 8.4 °C and 9.3 °C [44]. Nine of the sites are former agricultural sites whereas the other five are former brown coal open-cast mining areas. These two site-types were chosen as their former land use clearly differs regarding their soil characteristics. Former mining soils are relatively acidic due to carboniferous and sulfur-containing substrates, as well as high iron and aluminum concentrations. These sites have low soil pH and low nutrient concentrations. In contrast, former agricultural soils have higher soil pH (>5.5) and nutrient availability caused by fertilization in the past [45]. Data regarding former utilizations, stand age, planting distance, harvest periods, and regional distinctions were provided by the owner or manager.

Table 1. Site description including federal states, location, longitude (long), latitude (lat), elevation above sea level (ASL), mean annual temperature (MAT), mean annual precipitation (MAP), former utilization, age, and rotation.

Site	Federal States *	Location	long (°E)	lat (°N)	ASL (m)	MAT (°C)	MAP (mm)	Former Utilization	Age (years)	Rotation
BE	MV	Bennin	10°51'49"	53°27'45"	35	8.9	589	Agriculture	8	0
BG	BB	Blumberg	14°10'24"	53°12'25"	22	8.8	528	Agriculture	3	2
BH	MV	Buchholz	12°38'9"	53°15'34"	64	8.6	632	Agriculture	2	1
CA	BB	Cahnsdorf	13°45'54"	51°52'18"	63	9.4	555	Agriculture	5	0
DA	BB	Grunow-Dammendorf	14°25'5"	52°8'26"	70	9.4	555	Agriculture	3	1
GU	BB	Gumtow	12°14'11"	52°59'46"	61	7.4	495	Agriculture	2	1
HM	ST	Hohenmölsen	12°6'1"	51°10'23"	149	9.4	525	Mining	2	0
KL	BB	Klein Loitz	14°30'57"	51°36'35"	140	9.4	555	Agriculture	2	3
LH	BB	Lauchhammer	13°50'57"	51°32'20"	111	9.4	555	Mining	8	0
PA	BB	Paulinenaue	12°43'52"	52°39'44"	31	7.4	495	Agriculture	3	1
RA	BB	Ragow	13°33'7"	52°18'8"	41	9.8	584	Mining	7	0
RM	ST	Röblingen	11°42'42"	51°25'57"	149	9.4	525	Mining	7	0
WA	BB	Wainsdorf	13°29'40"	51°24'50"	92	9.3	671	Agriculture	1	2
WZ	BB	Welzow	14°14'7"	51°33'32"	123	9.4	555	Mining	1	1

* BB: Brandenburg, ST: Saxony-Anhalt, MV: Mecklenburg-Western Pomerania.

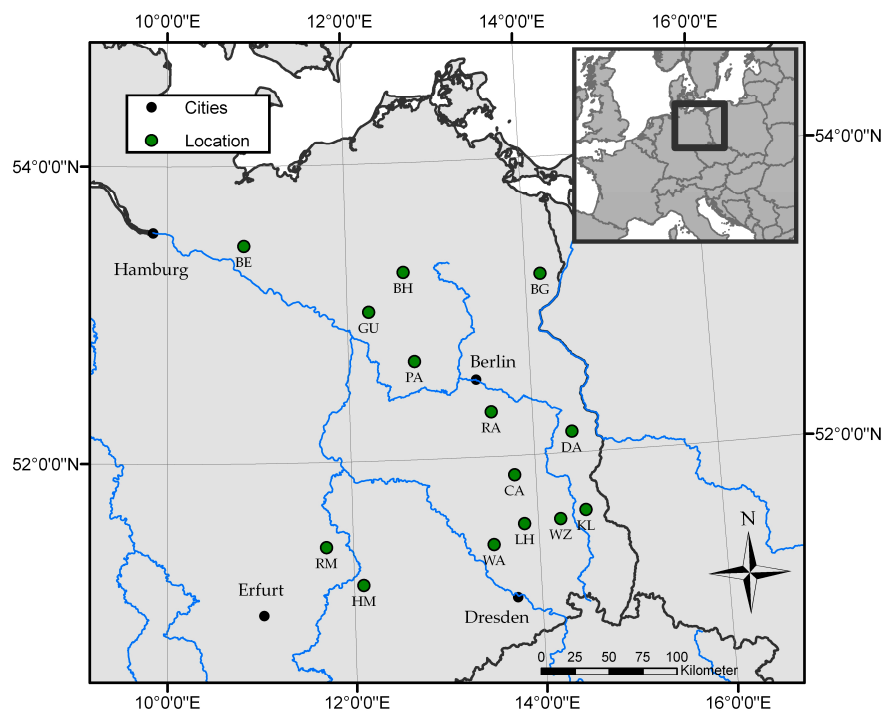


Figure 1. Map of the study area in Europe, indicating the 14 sampling sites (locations) in Germany of *Robinia pseudoacacia* L. [46]. For an overview of site abbreviations, see Table 1.

2.2. Field Data Collection and Calculation

Tree parameters were collected between November 2016 and January 2017, measuring leafless trees in 62 sample plots. In total, 9729 black locust trees were investigated. Of these, 6407 trees were surveyed in Brandenburg, 1876 trees in Saxony-Anhalt, and 1446 trees in Mecklenburg-Western Pomerania. To all investigated stands, the same procedures were applied by the same investigator to avoid site-specific biases. The calculation of the necessary sample size [47] (p -value < 0.05) of 427 trees per stand was achieved by taking a sample surface (600 diameter) of the plantations in Röblingen (Saxony-Anhalt). Furthermore, based on a calculated raster (uniform distribution on each site map) three plots per study area with more than 150 trees were measured. In two areas, the measurement of only one plot was feasible (size of the areas < 0.6 ha). In the 14 study sites, the planting distance

and plant loss were different. Therefore, the radius per plot varied in each stand; minimum 4 m till maximum 14 m.

The diameter at breast height (DBH) and root collar diameter (RCD) were measured at more than 150 trees per sample plot. Each sprout of the stump was measured as an individual tree. DBH was measured with a diameter measurement tape at a height of 1.3 m. To allow comparability with previous studies [20,41] and to account for the different conditions at the 62 sample plots (core and stump growth), RCD was consistently measured at a height of 0.1 m. The basal area (BA) per tree was calculated using RCD in the circular area formula. As an index of competition, the percentile of the BAs frequency distribution (PCT) [48,49] was used. The classification of individual trees was calculated by dividing the tree-specific RCD from the stand average of RCD (d/dg).

The local stand density index (SDI) by Reineke [50] was calculated to compare competition levels between the fourteen sample sites. SDI was calculated with the number of plants (NoP) in each study site. Furthermore, dg represents the average value of RCD in the 14 study sites. The value of the exponent, -1.605 , was used according to the generic stand density rule by Reineke [16,17,50,51]. Reineke [50] analyzed the stand density index for evenly-aged forests with different stand densities (under- and over-stocked). For small trees like in this study, it was possible to calculate the SDI by using a numerator of 5 cm (mean tree diameter).

$$SDI = NoP \left(\frac{5}{dg} \right)^{-1.605} \quad (1)$$

After measurement of diameter (RCD, and DBH), trees were categorized. Hence, for 25 trees per plot, representative of the whole diameter spectrum (categories), the following parameters were additionally measured: tree height, height to crown base, fresh aboveground woody biomass after cutting 10 cm above soil surface (kg, overall total weight 4.5 tones), biomass aliquot (kg, overall total weight 614 kg), and biomass after dehydration (g). Tree height (H) and crown base height (CB) [distance from the lowest primary branch to the top of the crown] were tape measured after cutting the tree. Similar to d/dg , individual trees in the stand were classified, by dividing the tree-specific height from the average stand height (h/hg). The classification of the value of the average height of predominant trees (h_{100}) and age (Bon) was calculated by employing the yield table by Lockow and Lockow [37] used for *Robinia pseudoacacia* L. Lockow and Lockow [37] analyzed trees with a minimum age of five years; thus, the extrapolation for younger trees was necessary. To express tree slenderness, their height was divided by diameter (h/d). Biomass after cutting and biomass after dehydration were measured by using an electronic scale with a spring balance (precision ± 1 g). For each sample, a representative, plot-related aliquot of shredded tree biomass was dried for 48 h in the laboratory at 103.5°C (DIN 52183 [52]) until a constant mass was reached [20,34–36,53,54]. This aliquot was used to estimate the total value of dry woody biomass for each sample.

2.3. Climate Data

Data of temperature, precipitation, and daylight hours (sun) were provided by the German weather service DWD [44] for the period of 1965 to 2015 (50 years) of eight weather stations (Angermünde, Berlin Tempelhof, Cottbus, Dresden-Klotsche, Leipzig-Halle, Marnitz, Neuruppin, Schwerin) in close proximity to the sampled stands. Based on these climate data, the De Martonne aridity index (DMI) [55] was calculated.

$$DMI = \frac{\text{Precipitation}}{\text{Temperature} + 10} \quad (2)$$

The aridity index of De Martonne is useful for assessing the relationships between water supply and tree growth [11,56]. Also, an important qualitative climatic variable for plants is the climatic water balance (CWB), described as the difference between precipitation and potential evapotranspiration [57].

2.4. Statistical Analysis, Allometric Modelling and Total Biomass Production

At first, a Pearson correlation matrix [58] was calculated to assess correlations between mean stand dry biomass and all acquired variables averaged per stand, such as RCD, DBH, BA, H, h/d, CB, Age, NoP, SDI, Bon, DMI, CWB, Prec, Temp, and Sun.

Linear regression models were calculated to test the structure of individual tree data. However, linear models do not represent the real nature of biomass production. The correlation between growth variables of single trees can be described with an allometric function, as given in Equation (3) [59]:

$$BM = ax^b \quad (3)$$

where a and b are scaling coefficients and x is the independent and explanatory variable. All regressions were undertaken using natural logarithmic transformation (Equation (4)) of the tree dimensions, in line with previous work done by Pretzsch et al. [56], Böhm et al. [20], Annighöfer et al. [34,42], and Stankova et al. [35]:

$$\ln(y) = a + b * \ln(x) \quad (4)$$

A nonlinear least square regression was employed to obtain estimates for the coefficients a and b . Mixed models by Fisher [60] allow the use of different data frames within one model:

$$\ln(y)_{ijk} = a_0 + a_1 \ln(x)_{ijk} + b_i + b_{ij} + \varepsilon_{ijk} \quad (5)$$

Here, the three different subdivisions are location (i), circle of the samples (j), and the individual tree (k). x represents tree features, competitive indices, and climate parameters (altogether 17 variables were tested). Additionally, competition variables like h/hg , d/dg , and PCT were integrated into the tree individual allometric models to predict biomass, as supplement to variables of the correlation matrix (tree- and climate parameter). Since it was not possible to obtain DBH for trees smaller than 1.3 m, RCD was fitted as the predictor variable. To quantify model performance and to compare among models, an analysis of variance (ANOVA) was performed. The standard error as the standard deviation of the sample distribution, p -value, as well as confidence intervals of every variable were derived. Conditional coefficient of determination (R^2) [61], variance of the residuals, the Akaike's Information Criterion (AIC) [62], and the Bayesian Information Criterion (BIC) [63] were used to select and rank among best fit models. For reasons of clarity, only the top four candidate models are presented.

Fixed effects were independent variables including tree diameter (RCD in cm) at 0.1 m, height (H in m), h/hg , and h/d . The following functional formulas were compared (Table 2, M_{01} – M_{04}).

Table 2. Allometric models to predict aboveground woody biomass, model number M_{01} – M_{04} , BM (Biomass dry), RCD (root collar diameter), H (height), h/hg (height divided by the mean BAs tree of height), and h/d (height divided by diameter). The letters a–e represent the parameters.

Name	Model
M_{01}	$\ln(BM) = a + b \ln(RCD) + \varepsilon$
M_{02}	$\ln(BM) = a + b \ln(RCD) + c \ln(H) + \varepsilon$
M_{03}	$\ln(BM) = a + b \ln(RCD) + c \ln(H) + d(h/hg) + \varepsilon$
M_{04}	$\ln(BM) = a + b \ln(RCD) + c \ln(H) + d(h/hg) + e(h/d) + \varepsilon$

To compare and cross-check the influence of different site conditions, dummy variables (a_3 and a_4) were used. Two different options for different growth exist in the data frame. These are related to former utilization (mining vs. agriculture) and type of growth (core vs. stump). The conditions are described by:

$$\ln(BM) = a_1 + a_2 \ln(RCD) + a_3x + a_4 \ln(RCD)x \quad (6)$$

To compare results for previous land use, zero (=0) was used for mining and one (=1) was used for agricultural site. In regards to growth, zero (=0) was used for core and one (=1) for stump growth. The percentage error (Equation (7)) was calculated by using M_{01} (Table 2) and M_{01t} (theoretical value using a special formula for core, stump, previous mining, and agricultural area) [59].

$$\%Error = \frac{(M_{01} - M_{01t})}{M_{01t}} * 100 \quad (7)$$

The analysis of total biomass production of the stands was calculated in tons per hectare and year [$\text{t to ha}^{-1} \text{ a}^{-1}$] for each stand individually with the measured data (allometric models are not included). The actual NoP and the area of each sample plot were used. Furthermore, the biomass of all trees in the sample plot was added up and extrapolated from plot size to one hectare [64]. NoP is not directly included in the allometric model. However, the NoP has a strong influence on the current total biomass production per hectare. To calculate aboveground woody biomass per year, the total biomass production at the time of measurements was divided by the stand individual age in years. To estimate the carbon stock, dry woody biomass estimates were multiplied by 0.5 following IPCC [65–67].

All analyses were performed in R [68], version R 3.3.2 GUI 1.68 (R Core Team, 2016), especially with the packages “stats” [69], “ape” [70], and “ggplot2” [71].

3. Results

3.1. Yield Data

Table 3 provides an overview on all obtained variables (RCD, DBH, tree height, crown base, wet tree biomass, and dry tree biomass) separated by former land use. Both land-use types expressed a minimum RCD and DBH of 0.1 cm, while the maximum RCD was at 20.2 cm and the maximum DBH was at 14.4 cm. The tree height ranged from 0.7 m to 9.9 m, wet tree biomass ranged from 0.01 kg to 51.81 kg, and dry tree biomass from 0.01 kg to 32.15 kg.

Table 3. Measured and calculated tree variables for each sampled site: growth *, root collar diameter (RCD), diameter at breast height (DBH), tree height, wet tree biomass, and dry tree biomass of *Robinia pseudoacacia* L., categorized by former utilization (# n_1 = 9729, ~ n_2 = 1550).

Site	Plots	# n_1	~ n_2	G *	# RCD [cm]	# DBH [cm]	~ Tree Height [m]	~ Wet Tree Biomass [kg]	~ Dry Tree Biomass [kg]
Mining		4311	700		4.2 (0.1–20.2)	3.1 (0.1–13.0)	4.4 (0.9–9.4)	3.6 (0.02–34.00)	2.36 (0.01–22.70)
HM	4	586	100	C	2.8 (0.4–7.8)	2.1 (0.2–4.3)	3.9 (1.6–6.5)	1.4 (0.07–4.98)	0.86 (0.04–2.87)
LH	6	952	150	C	5.4 (0.6–18.4)	4.2 (0.5–12.0)	5.3 (1.4–9.4)	4.8 (0.19–32.11)	3.17 (0.13–21.09)
RA	1	152	25	C	6.4 (1.3–15.1)	4.7 (0.8–9.3)	6.1 (4.7–7.2)	9.3 (2.57–23.40)	6.10 (1.71–15.74)
RM	9	1290	225	C	6.6 (0.5–20.2)	4.6 (0.4–13.0)	5.6 (1.5–9.0)	6.2 (0.10–34.00)	4.00 (0.06–22.70)
WZ	8	1331	200	S	1.4 (0.1–3.7)	1.1 (0.1–3.2)	2.6 (0.9–4.9)	3.6 (0.02–2.07)	0.19 (0.01–1.27)
Agriculture		5418	850		2.8 (0.1–16.7)	2.4 (0.1–14.4)	3.8 (0.7–9.9)	2.31 (0.01–51.81)	1.43 (0.01–32.15)
BE	3	446	75	C	7.3 (2.3–16.7)	5.7 (1.2–14.4)	6.4 (3.0–9.9)	8.68 (0.56–51.81)	5.29 (0.34–32.15)
BG	3	441	75	S	3.9 (1.2–9.7)	3.4 (1.0–7.8)	5.6 (2.0–7.9)	3.77 (0.24–21.79)	2.33 (0.14–13.62)
BH	6	1000	150	S	1.3 (0.1–4.0)	0.9 (0.1–2.8)	2.3 (0.8–4.0)	0.30 (0.01–1.77)	0.18 (0.01–1.10)
CA	3	470	75	C	4.7 (1.3–12.5)	3.4 (0.4–7.7)	5.2 (2.3–7.8)	3.60 (0.40–17.3)	2.28 (0.01–10.86)
DA	3	422	75	S	4.9 (1.0–11.3)	3.7 (0.9–8.6)	5.6 (2.0–9.4)	4.05 (0.08–19.59)	2.52 (0.04–12.06)
GU	8	1314	200	S	1.2 (0.1–4.4)	0.9 (0.1–3.1)	2.2 (0.7–4.3)	0.36 (0.01–2.35)	0.22 (0.01–1.41)
KL	3	478	75	S	3.4 (0.7–12.8)	2.7 (0.6–7.4)	5.6 (3.0–9.0)	3.03 (0.26–14.47)	1.90 (0.17–8.90)
PA	1	167	25	S	2.2 (0.3–6.5)	1.7 (0.6–4.5)	2.9 (0.7–5.0)	1.59 (0.16–3.79)	1.00 (0.10–2.39)
WA	4	680	100	S	1.6 (0.3–3.8)	1.0 (0.1–3.4)	2.5 (1.0–4.1)	0.69 (0.02–31.00)	0.44 (0.02–20.15)

* G: Growth; S: stump shoots; C: core growth; mean (minimum and maximum).

3.2. Correlation of Tree, Stand, and Climate Parameters

The correlation matrix (Table 4) indicated a strong correlation between dry biomass (BMD) with the DBH, RCD, H, and the age of the tree. The variables NoP and h/d showed a strong negative correlation with the BMD. All other variables expressed a weaker correlation with the BMD. The weakest observed correlation with the BMD was with precipitation, daylight hours (sun), the De

Martonne Index, and the climatic water balance. All tree individual variables correlate well with each other (BMD, RCD, DBH, H, and CB). Age correlates well with BMD, RCD, DBH, and height-diameter association (h_d) (>0.8). The SDI has the strongest association with CB (0.75). The correlation matrix illustrates a strong correlation (0.93) between NoP and h_d (tree slenderness), as well as NoP and the diameters RCD, and DBH. All climatic parameters show a weak correlation to all tree, stand, and climatic variables. Noticeable is the temperature, with a correlation (>0.4) to BMD, RCD, DBH, H, CB, SDI, Bon, and Prec. For the allometric modelling, the RCD ($DBH \times 1.42$) was only used as it is particularly applicable for young, small trees (<1.3 m tree height) and it is beneficial in cases, if forks are between 0.1 m and 1.3 m tree height.

Table 4. Correlation among mean tree and stand variables for *Robinia pseudoacacia* L.

	BMD	RCD	DBH	H	CB	Age	h_d	SDI	Bon	NoP	Prec	Temp	Sun	DMI	CWB
BMD	1	0.95	0.95	0.87	0.72	0.89	−0.82	0.32	−0.19	−0.79	0.03	0.43	−0.01	−0.18	0.28
RCD		1	0.99	0.92	0.82	0.91	−0.84	0.47	−0.07	−0.84	−0.04	0.47	0.08	−0.28	0.18
DBH			1	0.94	0.85	0.90	−0.82	0.49	−0.03	−0.84	−0.07	0.45	0.11	−0.30	0.15
H				1	0.91	0.75	−0.71	0.68	0.23	−0.80	−0.10	0.54	0.31	−0.37	0.10
CB					1	0.69	−0.59	0.75	0.22	−0.67	−0.30	0.45	0.32	−0.55	0.08
Age						1	−0.86	0.29	−0.39	−0.77	−0.06	0.30	−0.03	−0.21	0.11
h_d							1	−0.20	0.34	0.93	0.12	−0.19	0.10	0.23	−0.29
SDI								1	0.58	−0.29	−0.07	0.64	0.64	−0.38	0.02
Bon									1	0.08	0.09	0.48	0.59	−0.14	−0.18
NoP										1	0.29	−0.20	−0.08	0.42	−0.16
Prec											1	0.42	−0.12	0.90	−0.06
Temp												1	0.53	−0.02	0.11
Sun													1	−0.39	−0.46
DMI														1	−0.12
CWB															1

BMD: dry biomass, RCD: root collar diameter, DBH: diameter at breast height, H: height, CB: crown base, Age: tree age, h_d : height-diameter association, SDI: stand density index, Bon: classification, NoP: number of plants, Prec: Precipitation, Temp: temperature, Sun: sun duration, DMI: De Martonne Index, and CWB: climatic water balance.

3.3. Allometric Models

RCD had the strongest association on BMD (M_{01}). Model M_{02} showed the best conditional coefficient of determination ($cR^2 = 0.953$) of all models and is a combination of RCD and tree height. Furthermore, M_{04} was the minimum adequate model based on AIC, BIC, and variance. That is, a combination of RCD, H, h/hg , and h/d (Table 5). According to the models, the influence of environmental variables on the BMD was negligible. The temperature influenced the model, but did not create a better model fit. The confidence interval of biomass estimates of Model M_{01} is presented in the Appendix A (Figure A1). The axes (x - and y -axis) of the confidence interval are log-transformed. Consequently, increasing RCD (or tree height) implies increasing the confidence interval and inaccuracy of the estimated values (Model M_{01} – M_{04}).

Site conditions (former mining sites versus agricultural sites) and growth type (core versus stump) influenced biomass prediction from the allometric equation. The analysis by using dummy variables (Equation (10)) showed significant differences between different land use and growth types (Tables 6 and 7, Figure 2). Overall, significance was obtained in the analysis of the BM versus RCD, comparing former mining areas with former agricultural areas (p -value < 0.05). Significant differences were also shown with the dummy variables in the comparison of BM with H (p -value < 0.001). The results also showed significant differences in woody biomass production between the growth types (core versus stump) in the categories BM and RCD, as well as BM and H (p -value < 0.001).

Figure 2 presents the nonlinear least square relationship and allometric relationship of double-logarithmic representation between RCD (cm) and aboveground woody biomass (kg), tree height (m) and aboveground woody biomass [kg] separated by previous land utilization (agriculture and mining) and growth (core and stump). In all categories, the equations and biomass estimates are very similar if the trees are small (RCD, and tree height). Larger trees show different

aboveground woody biomass accumulation between agriculture and mining, core and stump. In previous agricultural areas, trees produce at the equal RCD more aboveground woody biomass than in previous mining areas. At the same tree height, the accumulated biomass is higher in mining areas. Moreover, by comparing the equations of core and stump, stumps accumulated more biomass at the equal RCD and core at the identical tree height.

Table 5. Comparison among the top four candidate models.

Model	Variable	Coefficient	Estimate	SE	<i>p</i> -Value	cR^2	AIC	Variance	Confidence Interval	
									2.5%	97.5%
M_{01}	Intercept	a	−2.65499	0.0436	<0.001	0.9353	1890	0.2061	−2.7404	−2.5696
	RCD	b	2.29325	0.0239	<0.001				2.2464	2.3401
M_{02}	Intercept	a	−3.50576	0.0649	<0.001	0.9532	1466	0.1572	−3.6331	−3.3784
	RCD	b	1.52190	0.0403	<0.001				1.4429	1.6008
	H	c	1.20956	0.0541	<0.001				1.1035	1.3156
M_{03}	Intercept	a	−3.40775	0.0794	<0.001	0.9492	1437	0.1567	−3.5634	−3.2521
	RCD	b	1.50926	0.0399	<0.001				1.4309	1.5876
	H	c	0.58536	0.1183	<0.001				0.3532	0.8175
	h/hg	d	0.63665	0.1063	<0.001				0.4280	0.8452
M_{04}	Intercept	a	−3.69133	0.0930	<0.001	0.9510	1419	0.1528	−3.8736	−3.5090
	RCD	b	1.94216	0.0961	<0.001				1.7536	2.1307
	H	c	0.27772	0.1340	0.0383				0.0149	0.5405
	h/hg	d	0.53084	0.1056	<0.001				0.3236	0.7380
	h/d	e	0.26262	0.0535	<0.001				0.1576	0.3676

RCD: root collar diameter, H: height, h/hg: stand normalized height, h/d: slenderness, SE: standard error of the estimate, *p*-value, cR^2 : conditional coefficient of determination, AIC: Akaike's Information Criterion, variance, and confidence interval.

Table 6. Comparing different sites and growth conditions by using dummy variables of power regression in the mixed models, with woody biomass (BM) as the response variable and the tree variables root collar diameter and height as predictor variables, for mining, agriculture, core, and stump (Equation (10)). The table below shows the parameter (a_1, a_2, a_3, a_4), estimates, standard error (SE), and *p*-value ($n = 1550$).

Model	Variables	Parameter	Estimate	SE	<i>p</i> -Value
Mining vs. Agriculture	BM and RCD	a_1	−2.73527	0.0849	<0.001
		a_2	2.36960	0.0377	<0.001
		a_3	0.14295	0.1054	0.2
		a_4	−0.13925	0.0494	<0.05
Mining vs. Agriculture	BM and H	a_1	−4.90281	0.0696	<0.001
		a_2	3.35715	0.0478	<0.001
		a_3	0.42611	0.0846	<0.001
		a_4	−0.37694	0.0602	<0.001
Core vs. stump	BM and RCD	a_1	−2.94658	0.0862	<0.001
		a_2	2.47837	0.0405	<0.001
		a_3	0.41101	0.1012	<0.001
		a_4	−0.30298	0.0513	<0.001
Core vs. stump	BM and H	a_1	−4.12556	0.0970	<0.001
		a_2	2.91852	0.0590	<0.001
		a_3	−0.42888	0.1070	<0.001
		a_4	0.02504	0.0711	0.725

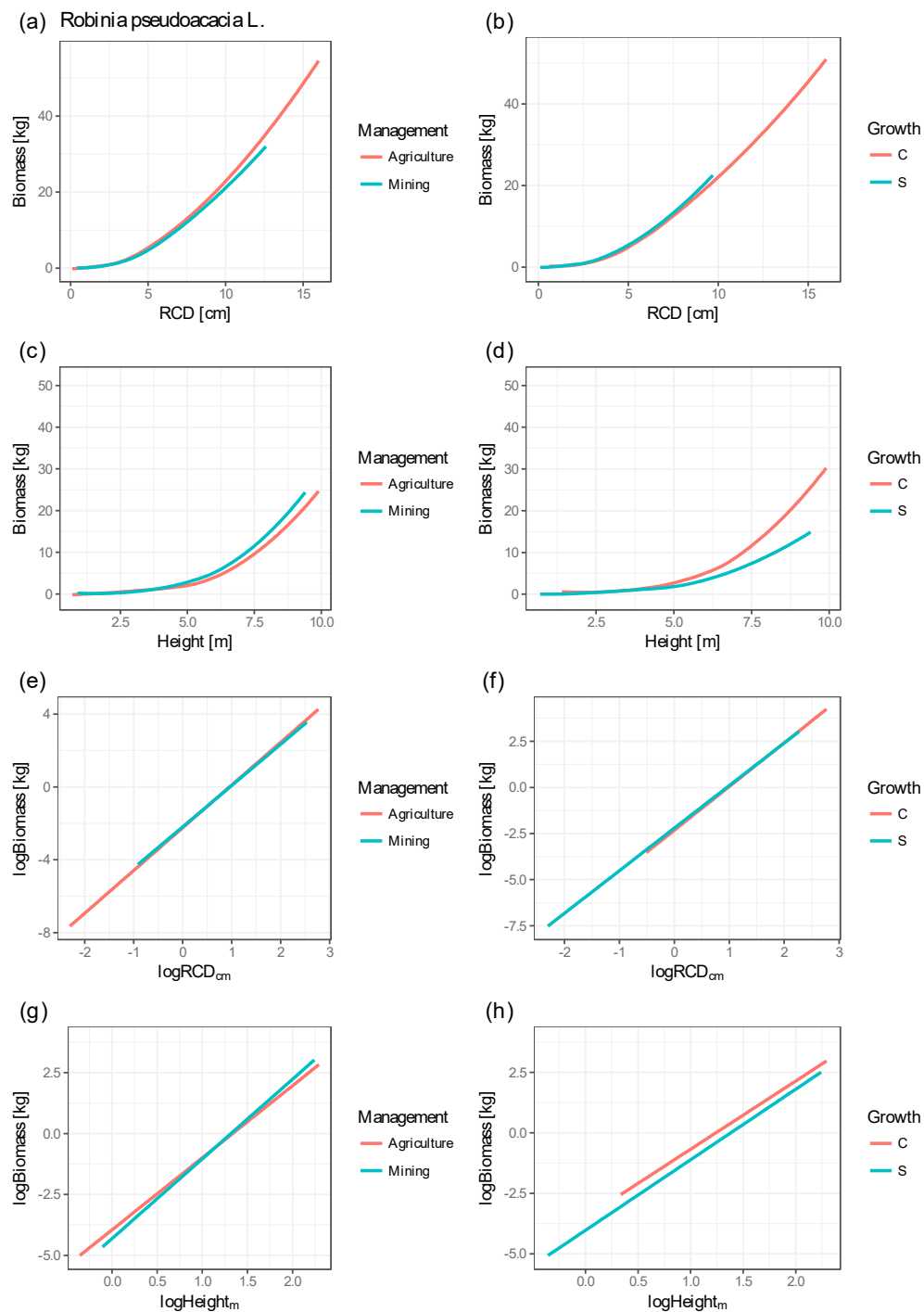


Figure 2. Allometric nonlinear least square relationship (a–d) and allometric relationship of double-logarithmic (e–h) representation between RCD (cm) and aboveground woody biomass (kg) (a,b,e,f), tree height (m) and aboveground woody biomass (kg) (c,d,g,h) separated by previous land utilization (Agriculture and Mining) and growth (C = Core and S = Stump). The statistical characteristics of the regression lines are presented in Tables 6 and 7.

Table 7. Comparison of different former utilization and growth type models, by using the value of 4.7 cm and 9.0 cm for RCD and 5.2 m and 7.3 m for H, Min = Mining, Agr = Agriculture, C = Core, and S = Stump.

Figure 3	Explanatory Variable	Value	Min (kg)	Agr (kg)	Min–Agr (kg)	Hectare (to)	C (kg)	S (kg)	C–S (kg)	Hectare (to)
(a), (b)	RCD (cm)	4.7	2.449	2.362	0.087	0.722 [#]	2.478	2.247	0.231	1.923 [#]
		9.0	11.713	10.054	1.659	3.318 *	12.292	9.164	3.128	6.256 *
(c), (d)	H (m)	5.2	1.648	1.677	0.029	0.244 [#]	1.991	1.573	0.418	3.480 [#]
		7.3	4.305	4.663	0.358	0.717 *	5.185	4.425	0.760	1.520 *

[#] 8333 NoP, * 2000 NoP (assumed values).

In Table 7, two selected values, arithmetic mean and 75% quartile (upper quartile, 25% of the largest values in the data set) of all stands, represent the differences for an individual tree (Min–Agr, C–S) and a one-hectare plantation of black locust woody biomass (Hectare). By using biomass equations with a RCD of 4.7 cm (mean value of all stands), the difference between mining and agriculture was at 0.09 kg per tree and between Core and Stump 0.23 kg per tree. Calculations with a RCD of 9.0 cm (75% quartile of all stands) showed a difference of 1.66 kg per tree (mining vs. agriculture) and 3.13 kg per tree (core vs. stump). Consequently, this is a difference of 3.3 tons per hectare (mining vs. agriculture) and 6.3 tons per hectare (core vs. stump) when assuming 2000 trees per hectare. Regarding the comparison of height (mining vs. agriculture, core vs. stump), the maximum difference with a height of 7.3 m is 0.76 kg per tree (Table 7). The percentage error for an RCD of 5 cm, when applied to M_{01} , is -2.4% for C_{01} (core), 9.7% for S_{01} (stump), 3.9% for Agr_{01} (agriculture), and -0.9% for Min_{01} (mining). Further details on all allometric models—mining, agriculture, core, and stump—are concluded in the Appendix A (Table A1).

3.4. Absolute Wood bioMass Productivity

The absolute stand biomass productivity per hectare of juvenile black locust trees in the analyzed study stands is presented in Table 8. The minimum dry biomass productivity was found to be 1.0 to $ha^{-1} a^{-1}$ and the maximum was at 13.8 to $ha^{-1} a^{-1}$. Differences in wet biomass ranged from a minimum of 1.6 to $ha^{-1} a^{-1}$ to a maximum of 22.1 to $ha^{-1} a^{-1}$. Carbon sequestration per hectare and year ranged from 0.5 tons to 6.9 tons. The mean annual carbon storage per hectare and year is 2.9 tons.

Table 8. Ranking of the study sites based on aboveground woody biomass productivity (to $ha^{-1} a^{-1}$).

Rank	Site	Former Utilization	n	Age	Biomass Wet		Biomass Dry		Carbon Sequestration (to $ha^{-1} a^{-1}$)
					(to ha^{-1})	(to $ha^{-1} a^{-1}$)	(to ha^{-1})	(to $ha^{-1} a^{-1}$)	
1	KL	Agriculture	75	2	44.2	22.1	27.6	13.8	6.9
2	BG	Agriculture	75	3	48.6	16.2	30.0	10.0	5.0
3	DA	Agriculture	75	3	44.7	14.9	27.9	9.3	4.7
4	HM	Mining	100	2	23.8	11.9	14.4	7.2	3.6
5	CA	Agriculture	75	5	55.5	11.1	35.5	7.1	3.6
6	WZ	Mining	200	1	9.8	9.8	6.0	6.0	3.0
7	WA	Agriculture	100	1	9.5	9.5	5.9	5.9	3.0
8	RM	Mining	225	7	55.3	7.9	37.1	5.3	2.7
9	LH	Mining	150	8	43.2	5.4	28.0	3.5	1.8
10	RA	Mining	25	7	34.3	4.9	22.4	3.2	1.6
11	BE	Agriculture	75	8	39.2	4.9	24.0	3.0	1.5
12	BH	Agriculture	150	2	7.6	3.8	4.6	2.3	1.2
13	GU	Agriculture	200	2	7.2	3.6	4.2	2.1	1.1
14	PA	Agriculture	25	3	4.8	1.6	3.0	1.0	0.5

4. Discussion

4.1. Correlation of Stand Parameter and Allometric Biomass Models

Although numerous studies exist dealing with the biomass productivity of black locust [19,20,31,34,35,37,38,64,72,73], the existing tools are very limited for quantifying the behavior of black locust in SRC in previous brown coal mining and agricultural areas. However, the benefit of *Robinia pseudoacacia* L. in SRC for our society, ecology, and economy lies in biomass and energy production, as well as carbon sequestration. By comparing stand parameters on the basis of a correlation matrix, we found that most important factors to explain the variability of dry biomass were RCD, DBH, age, height, and h/d association. Dahlhausen et al. [17] reported a significant correlation between SDI and biomass production in young oak stands (*Quercus robur*). Stand density index in this study did not show this influence because it is a collective SDI and not an individual tree SDI.

The most frequently used independent variables for tree biomass equations are RCD, DBH, H, and age (tree stem parameter), particularly for black locust [19,20,31,34–38]. Modelling by employing mixed models, as used by Forrester et al. [74], Grote et al. [75], and Pretzsch et al. [76] has the advantage of being applicable at different geographical and differently structured sites. In this study, RCD had the strongest correlation with BMD (M_{01}). Model M_{02} (being based on RCD and H) was the top equation with respect to the coefficient of determination (R^2). Furthermore, in regards to AIC [77] and variance, model M_{04} (being based on RCD, height, h/hg, and h/d) performed best. Given the rather similar model performance, practical users can choose between M_{02} and M_{04} . Both models provide similar estimates. It is recommended to use the simpler over the more complex model, but for the sake of completeness, it was decided to present both models here. The average temperature was the climatic parameter with the strongest influence among climatic parameters. However, it did not improve model fit significantly. The confidence intervals of biomass estimates show for all models that the results are as accurate as possible, if the variables RCD and tree height are as small as possible. The inaccuracy increases with increasing explanatory variables (RCD, tree height).

A comparison with three other biomass equations from the literature revealed that the allometric model M_{01} had the comparably lowest slope (Figure 3). This is probably related to the differing representations of the studies considered. That is, Lange et al. [41] calculated a biomass equation for young black locust trees in an ecosystem forest which featured a lower number of planted trees per hectare (2000–3000) compared with typical SRCs (8000–10,000 [7]). Compared to our equation, the differences at RCD 10 cm were 15.0 kg per tree which probably reflects the different stature of trees under differing stand densities. The study by Annighöfer et al. [42] analyzed seedlings of 19 tree species, including *Robinia pseudoacacia* L. ($n = 238$). Compared to our study, there is a difference of 1.3 kg per tree for a RCD of 3.0 cm. The maximum RCD reported by Annighöfer et al. [43] was at 3.9 cm, but in our study the maximum RCD was at 20.2 cm, which was a large deviation in the analyzed RCD spectrum. The equation presented by Böhm et al. [20] expressed the highest similarity to our model. That is, up to the RCD of 7 cm, the curves were equal. However, at RCD of 13.0 cm, biomass prediction from Böhm et al. [20] was 10.0 kg per tree higher compared to our model. The relatively high similarity is probably due to the fact that Böhm et al. [20] analyzed black locust on reclaimed soil in a former open-cast mining area in the Welzow energy forest (EEW), which is also one of the sites investigated in this study. The similarity is influenced by the subsample effect.

Land management in the past significantly influenced biomass production in the presence. The allometric models M_{01} – M_{04} are based on the complete data set. Therefore, to obtain more precise results, the integration of the former land utilization (mining or agriculture) and growth by core or stump will improve model predictions. In terms of energy production in the past and present, Germany exhibits several former open cast lignite mines. Just recently, these disturbed landscapes are being restored and recultivated [78]. Within this context, black locust was planted as leguminous and modest tree species [9]. In this study, it was possible to compare previous mining

with previous agricultural areas. In agricultural areas, the results showed that aboveground woody biomass is higher at the same RCD, so presumably the plants concentrate on vertical growth and biomass allocation. An assumption of this fact is that biomass accumulation at the equal RCD is higher if the tree height growth is superior. A reason for those results could be that tree height growth is strongly influenced by soil and site conditions. In this study, it seems that trees in former agricultural sites have better height growth than trees in nutrient poor former mining sites. In former mining areas, the biomass at the same tree height is higher than in agricultural areas. Probably, the tree growth in former mining areas is reinforced on radial growth and biomass allocation. Diameter growth is more influenced by competition for light, water, and nutrients in even-aged stands. The connection between diameter growth and competition is also indicated by the strong correlations between numbers of plants and RCD (DBH) in Table 4. Practitioners can apply the global models, but with increasing RCD and tree height the differences are not negligible. For example, the calculation of biomass with allometric models for mining and agriculture (Appendix A, Table A1) showed a difference at an RCD of 9.0 cm of 3.3 tons per hectare (NoP 2000). In detail, the reasons for this different behavior are not yet clear and consequently more research is needed on the ecological reactions of black locust to different environmental conditions. However, it seems possible that a higher nutrient load in former agricultural sites may affect the relatively stronger vertical growth.

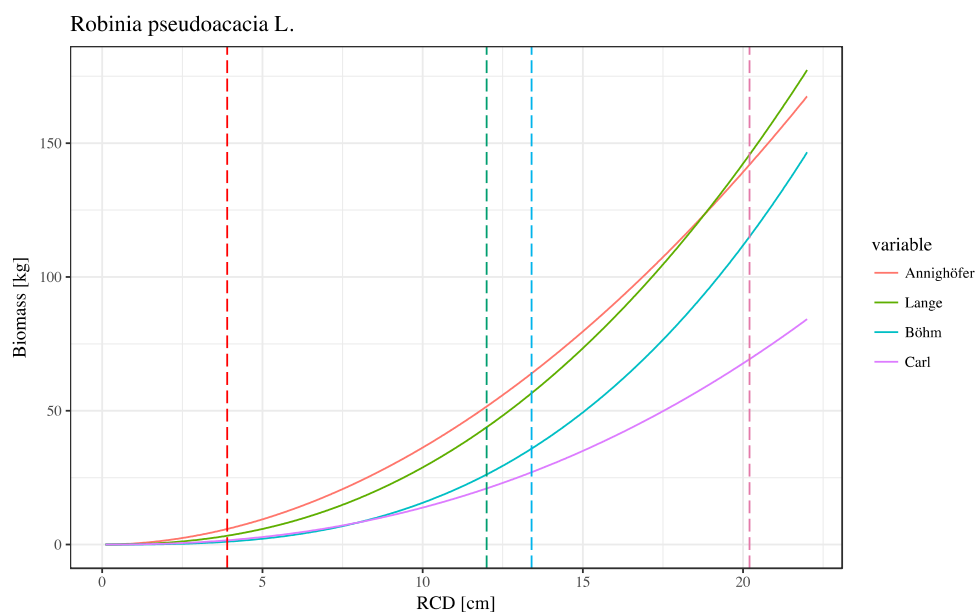


Figure 3. Biomass equations comparing *Robinia pseudoacacia* L. biomass to RCD of different investigations, including the current study M_{01} , Lange et al. [41], Annighöfer et al. [42], and Böhm et al. [20]. The dashed line illustrates the maximum RCD, which was incorporated into the biomass equations.

In Germany, it is common to plant SRC in short-, medium-, or long-term rotation systems [6,7] independent of the planned harvesting periods from the local manager. After harvesting (first, second, etc., rotation), *Robinia pseudoacacia* L. resprouts from the stumps with a varying multiple number of sprouts. These sprouts of one stump compete with each other for resources, especially light. All in all, significant differences were observed between the growth types core (planted trees before harvesting, zero rotation) and stump (after harvesting, first, second, etc., rotation). Stumps have a higher slope in regression of RCD to biomass. Therefore, at the same RCD, sprouts have a higher aboveground woody biomass accumulation in the analyzed areas. Multiple sprouts on the same stump are in closer proximity to each other than sprouts from different stumps. Hence, the assumption is that competition for light among sprouts on the same stump is more intense than among sprouts from different stumps.

and trees growing by the core. Competition for light usually results in taller, more slender stems because height growth is less negatively influenced than radial growth. The multiple sprouts per stump share a common root system. Therefore, they may have different abilities to compete for the shared water and nutrient supply. In general, taller and faster growing sprouts likely get more of the resources than shorter, shaded, and slower growing sprouts. All in all, in consideration of certain parts of the shoot and certain parts of the root system, more vigorous sprouts likely provide more carbohydrates to the roots that provide them water and nutrients, creating a positive feedback that allows the larger sprouts to obtain a larger part of the shared soil resources. Cores have a higher slope in regression of height to biomass. Therefore, at the same height, cores have a higher aboveground woody biomass accumulation in the analyzed areas. Probably, cores focus on radial growth and biomass allocation. Within-stump competition is not present in trees growing by core. Core trees have only a single stem per root system. However, cores must allocate a higher proportion of their fixed carbon to building their root system compared to sprouts which already have an existing root system. This would certainly influence allocation to above-ground biomass production. The difference between core and stump is even more concise than in areas affected by former mining and agriculture usage. Here, at RCD 9.0 cm, the aboveground woody biomass differs by 6.3 tons per hectare (NoP 2000). These differences are probably related to different levels of competition between core stands and stump stands, but further research is needed to get a better understanding of the details of this behavior.

Growth differences exist between growth in previous mining and agricultural areas, core and stump growth. The reason for these growth differences should be analyzed in further studies dealing with the ecological effects of the different initial conditions (previous utilization and growth) to black locust. The global equations (M_{01} – M_{04}) are applicable, but if the previous utilization and growth conditions are known, specific models can be used (agriculture, mining, core, and stump). Overall, the woody biomass mixed models offer the possibility to calculate the biomass of black locust in SRC for practitioner, foresters, researcher, students, and other interested stakeholders. A biomass calculator as a tool for *Populus* in SRC exists [53,54,79]. This study represents a scientifically grounded application for the tree species *Robinia pseudoacacia* L. in SRC.

4.2. Total Woody Biomass Productivity

Dry woody biomass production of the analyzed stands varied between 1.0 to $\text{ha}^{-1} \text{a}^{-1}$ and 13.8 to $\text{ha}^{-1} \text{a}^{-1}$. This fits well in the range of values reported elsewhere in literature. In Europe and North America, the biomass production of black locust ranges between 1.6–19.0 to $\text{ha}^{-1} \text{a}^{-1}$. The values of biomass per hectare and year of the described studies did not show changes in storage. The total biomass at the time of measurements was divided by the stand's individual age in years. Therefore, the calculated biomass storage per year is a mean annual biomass increment of the whole growing time. Grünwald et al. [64] reported that four former German mining areas expressed 3.0–10.0 to $\text{ha}^{-1} \text{a}^{-1}$ of core and stump growth, at a tree age of 3–14 years (stand age in this study ranged from 1–8 years). Their study found that the biomass production of black locust is higher than that of *Populus* and *Salix* in the same former mining areas [64]. Mirck et al. [38] estimated 6.9 to $\text{ha}^{-1} \text{a}^{-1}$ of black locust in a five-year-old agroforestry system. In Hungary, Redei et al. [32] calculated 3.0–6.0 to $\text{ha}^{-1} \text{a}^{-1}$ at five-year-old stems. In this study, the analyzed area in Cahnisdorf is the same age (five-years-old) and the production is 7.1 to $\text{ha}^{-1} \text{a}^{-1}$. Werner et al. [40] reported 19.0 to $\text{ha}^{-1} \text{a}^{-1}$ for trees of similar age, growing as stump stocks in agricultural landscapes. Peters et al. [39] found 5.0–6.0 to $\text{ha}^{-1} \text{a}^{-1}$ harvested in a six-year-old area in Germany. These values are comparable with the estimates of 5.3 to $\text{ha}^{-1} \text{a}^{-1}$ at the study site in Rößlingen analyzed in this study. In Austria, Müller et al. [33] reported 7.0–10.0 to $\text{ha}^{-1} \text{a}^{-1}$. In this study, three-year-old stands (Grunow-Dammendorf, Blumberg) show similar productivity (9.3–10.0 to $\text{ha}^{-1} \text{a}^{-1}$) to Müller's study. Stolarski et al. [19] found 1.6–5.4 to $\text{ha}^{-1} \text{a}^{-1}$ for four-year-old black locust stands in Poland. In the United States of America, Geyer et al. [26] described 11.7 to $\text{ha}^{-1} \text{a}^{-1}$ for two-year-old trees and 8.0 to $\text{ha}^{-1} \text{a}^{-1}$ for four-year-old black locust. The highest productivity in this study was measured in a two-year-old stand in Klein

Loitz with $13.8 \text{ to } \text{ha}^{-1} \text{ a}^{-1}$. A negative influence on biomass production in some of the study's sites could have been *Fusarium* fungi [80], and for other sites, it may have been grazing in the year of planting [81]. Positive influences on the tree growth could be a thorough soil surface preparation, plant care, and fostering [81]. The study sites of this study stored on average 2.9 tons of carbon per hectare and per year. Consequently, over the whole study area, black locust captured 974 tons of carbon per hectare and per year.

5. Conclusions

This study highlights the aboveground woody biomass production of *Robinia pseudoacacia* L. in SRC in northeast Germany. Overall, 17 variables (related to tree features, competition, and climate) were tested to calculate an individual tree's biomass. In this study, root collar diameter (RCD) had the strongest impact on dry biomass (M_{01}) and a model combining RCD with tree height (M_{02}) was the best model in regards to the coefficient of determination. Moreover, a significant influence of former land utilization (open cast mining areas versus agricultural fields) as well as the type of growth (core versus stump) was found. Up to now, only limited data are available for black locust growth in previous agricultural and mining areas in central Europe; more research dealing with allometry, functions, and ecology of black locust is required.

Furthermore, this study showed that dry woody biomass production ranged between 1.0 and $13.8 \text{ to } \text{ha}^{-1} \text{ a}^{-1}$, and the mean carbon storage was 2.9 tons of carbon per hectare and year. The models presented in this study provide local managers, foresters, and scientists with the opportunity to estimate productivity (biomass yield, energy potential) and consequently carbon sequestration of black locust in the field based on a low number of parameters. Allometric models, and biomass analysis fill the knowledge gap of yield production dealing with black locust in previous agricultural and mining areas.

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Author Contributions: Dirk Landgraf, Hans Pretzsch and Christin Carl initiated and planed the project; Christin Carl performed the experiments, analyzed the data together with Peter Biber and Allan Buras, and wrote the manuscript. Dirk Landgraf, Hans Pretzsch, Peter Biber and Allan Buras contributed by revising the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

AIC	Akaike Information Criterion
ANCOVA	Analyses of Covariance
ANOVA	Analyses of Variance
BA	Basal area
BIC	Bayesian Information Criterion
BM	Biomass (dry)
C	Core growth
CB	Crown base
CWB	Climatic water balance
DBH	Diameter at breast height
DMI	De Martonne Index
EEG	Erneuerbare-Energien-Gesetz (Renewable Energy law)
H	Height

NoP	Number of plants
PCA	Principle Component Analyses
PCT	Percentile of the basal area
RCD	Root collar diameter
S	Stump growth
SDI	Stand Density Index
SE	Standard error
SRC	Short rotation coppice
cR^2	Conditional coefficient of determination

Appendix A

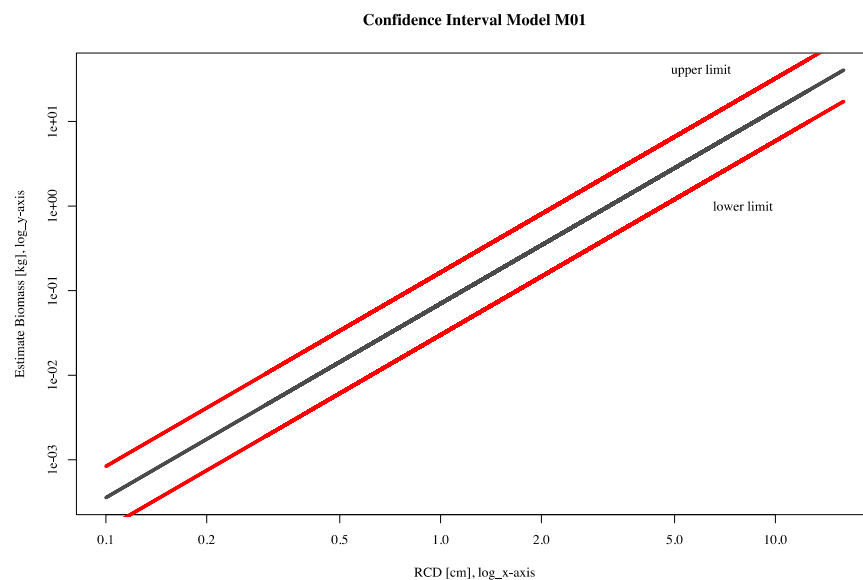


Figure A1. Confidence interval of Model M_{01} ; upper and lower limit. The x -axis and y -axis are log transformed.

Table A1. Variables, parameters, estimate, standard error (SE), p -value, conditional R^2 (cR^2), AIC, variance, and confidence interval (95%) for (ln) relationship of double-logarithmic of the mixed biomass equations $M_{01}H$, Mining (Min) M_{01} till M_{04} , Agriculture (Agr) M_{01} till M_{04} , Core (C) M_{01} till M_{04} , and Stump (S) M_{01} till M_{04} .

Model	Variable	Coefficient	Estimate	SE	p -Value	cR^2	AIC	Variance	CI	
									2.5%	97.5%
$M_{01}H$	Intercept	a	−4.2976	0.1141	<0.001	0.9025	2458	0.3566	−4.5215	−4.0737
		b	2.9365	0.0405	<0.001				2.8570	3.0161
Agr ₀₁	Intercept	a	−2.5903	0.0710	<0.001	0.9143	1249	0.2602	−2.7298	−2.4508
		b	2.2293	0.0357	<0.001				2.1591	2.2995
Agr ₀₁ H	Intercept	a	−4.4541	0.1454	<0.001	0.9001	1507	0.3784	−4.7396	−4.1687
		b	3.0152	0.0606	<0.001				2.8962	3.1341
Agr ₀₂	Intercept	a	−3.4829	0.1006	<0.001	0.9368	1089	0.2145	−3.6805	−3.2853
	RCD	b	1.4897	0.0633	<0.001				1.3653	1.6141
	H	c	1.2090	0.0895	<0.001				1.0334	1.3846
Agr ₀₃	Intercept	a	−3.4077	0.07935	<0.001	0.9533	1468	0.2139	−3.5634	−3.2520
	RCD	b	1.5092	0.0399	<0.001				1.4309	1.5876
	H	c	0.5853	0.1183	<0.001				0.3532	0.8175
	h/hg	d	0.6366	0.1063	<0.001				0.4280	0.8452

Table A1. Cont.

Model	Variable	Coefficient	Estimate	SE	p-Value	cR^2	AIC	Variance	CI	
									2.5%	97.5%
Agr ₀₄	Intercept	a	−3.6913	0.0929	<0.001	0.9510	1419	0.2056	−3.8737	−3.5090
	RCD	b	1.9421	0.0961	<0.001				1.7536	2.1307
	H	c	0.2777	0.1339	0.0383				0.0149	0.5405
	h/hg	d	0.5308	0.1056	<0.001				0.3236	0.7380
	h/d	e	0.2626	0.0535	<0.001				0.1576	0.3676
Min ₀₁	Intercept	a	−2.8322	0.0726	<0.001	0.9626	468	0.1353	−2.9749	−2.6894
	RCD	b	2.4089	0.0330	<0.001				2.3441	2.4737
Min _{01H}	Intercept	a	−4.1685	0.2906	<0.001	0.9132	765	0.3150	−4.7393	−3.5976
	H	b	2.8313	0.0547	<0.001				2.7238	2.9389
Min ₀₂	Intercept	a	−3.5994	0.0482	<0.001	0.9761	175	0.0768	−3.6942	−3.5046
	RCD	b	1.5952	0.0407	<0.001				1.5152	1.6754
	H	c	1.1811	0.0583	<0.001				1.0665	1.2957
Min ₀₃	Intercept	a	−3.4077	0.0793	<0.001	0.9492	1436	0.0757	−3.5634	−3.2521
	RCD	b	1.5092	0.0399	<0.001				1.4309	1.5876
	H	c	0.5853	0.1183	<0.001				0.3532	0.8175
	h/hg	d	0.6366	0.1063	<0.001				0.4280	0.8452
Min ₀₄	Intercept	a	−3.6913	0.0929	<0.001	0.9510	1419	0.0757	−3.8737	−3.5089
	RCD	b	1.9421	0.0961	<0.001				1.7536	2.1307
	H	c	0.2777	0.1339	0.0383				0.0149	0.5405
	h/hg	d	0.5308	0.1056	<0.001				0.3236	0.7380
	h/d	e	0.2626	0.0535	<0.001				0.1576	0.3676
C ₀₁	Intercept	a	−2.9081	0.0672	<0.001	0.8759	832	0.2073	−3.0400	−2.7760
	RCD	b	2.4654	0.0404	<0.001				2.3859	2.5448
C _{01H}	Intercept	a	−3.9640	0.1332	<0.001	0.8111	1077	0.3223	−4.2256	−3.7024
	H	b	2.8220	0.0596	<0.001				2.7048	2.9391
C ₀₂	Intercept	a	−3.6598	0.0769	<0.001	0.9092	625	0.1497	−3.8108	−3.5088
	RCD	b	1.6378	0.0628	<0.001				1.5144	1.7612
	H	c	1.1972	0.0755	<0.001				1.0490	1.3455
C ₀₃	Intercept	a	−3.4077	0.0793	<0.001	0.9492	1436	0.1473	−3.5634	−3.2521
	RCD	b	1.5092	0.0399	<0.001				1.4309	1.5876
	H	c	0.5853	0.1183	<0.001				0.3532	0.8175
	h/hg	d	0.6366	0.1063	<0.001				0.4280	0.8452
C ₀₄	Intercept	a	−3.6913	0.0929	<0.001	0.9510	1419	0.1473	−3.8737	−3.5089
	RCD	b	1.9421	0.0961	<0.001				1.7536	2.1307
	H	c	0.2777	0.1339	0.0383				0.0149	0.5405
	h/hg	d	0.5308	0.1056	<0.001				0.3236	0.7380
	h/d	e	0.2626	0.0535	<0.001				0.1576	0.3676
S ₀₁	Intercept	a	−2.5393	0.0863	<0.001	0.9168	1035	0.2040	−2.7086	−2.3700
	RCD	b	2.1639	0.0308	<0.001				2.1033	2.2244
S _{01H}	Intercept	a	−4.5737	0.1627	<0.001	0.9015	1383	0.3352	−4.8930	−4.2544
	H	b	3.0490	0.0560	<0.001				2.9390	3.1591
S ₀₂	Intercept	a	−3.4286	0.1025	<0.001	0.9405	845	0.1610	−3.6298	−3.2274
	RCD	b	1.4806	0.0538	<0.001				1.3750	1.5860
	H	c	1.1707	0.0793	<0.001				1.0151	1.3262
S ₀₃	Intercept	a	−3.4077	0.0793	<0.001	0.9492	1436	0.1610	−3.5634	−3.2520
	RCD	b	1.5092	0.0399	<0.001				1.4308	1.5876
	H	c	0.5853	0.1183	<0.001				0.3532	0.8175
	h/hg	d	0.6366	0.1063	<0.001				0.4280	0.8452
S ₀₄	Intercept	a	−3.6913	0.0929	<0.001	0.9510	1419	0.1528	−3.8737	−3.5089
	RCD	b	1.9421	0.0961	<0.001				1.7535	2.1307
	H	c	0.2777	0.1339	0.0383				0.0149	0.5405
	h/hg	d	0.5308	0.1056	<0.001				0.3236	0.7380
	h/d	e	0.2626	0.0535	<0.001				0.1576	0.3676

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Article 2: Key Drivers - Growth Partitioning

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Key drivers of competition and growth partitioning among *Robinia pseudoacacia* L. trees

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ABSTRACT

Competition for above- and below-ground resources depends on their availability and results in varied growth partitioning. This becomes clear as the supply and limitation of the main resources influence the slope of the size–growth relationship in stands. Nevertheless, growth partitioning among trees, especially among black locust (*R. pseudoacacia*) trees is not understood in sufficient detail. To scrutinize and estimate the mode of competition of *R. pseudoacacia*, this research analyzed 1333 trees in Germany in 10 study sites, consisting in total 27 sample plots, with similar climate but varying soil conditions. The stand age ranged from 2 to 32 years, with a diameter at breast height ranging from 0.6 to 29.1 cm. The main focus of the study was to evaluate the impact of nitrogen, phosphorus, and water supply on the competition mode of *R. pseudoacacia* by varying growth partitioning. We applied the size–growth relationship in a mixed-effects model with a random intercept and slope. Fixed effects were the basal area, phosphorus, water, the sunlight competition index, and the interactions between below- and above-ground resources. Site specific effects of the analyzed stands were quantified with the aid of the random effects. Depending on the supply and limitation of phosphorus and water, this study determined how the competition mode as well as the growth partitioning among *R. pseudoacacia* trees were influenced. Hence, if phosphorus and water availability increased, then the competition for above-ground resources (primarily sunlight) and the slope of the size–growth relationship increased. Large trees grew disproportionately more than smaller trees. If the available phosphorus and water decreased, then the competition for the below-ground resources increased and the slope of the size–growth relationship decreased – to be more flattened. Moreover, it was found that available nitrogen as a below-ground resource had no influence on the mode of competition. In summary, phosphorus was the most important nutrient and, together with water, was the main driver of growth partitioning among *R. pseudoacacia* trees.

1. Introduction

Growth partitioning among trees is influenced by the availability of above- and below-ground resources (Weiner, 1990) and is described as the “mode of competition” (Hara, 1993). Nevertheless, the competition mode is rarely included in forest research, especially nitrogen-fixing *R. pseudoacacia* stands are less investigated. However, by integrating detailed resource-limitation effects, growth partitioning may become more sensitive to prevailing conditions (Pretzsch and Biber, 2010) and resource availability. Furthermore, if resource limitation was better understood, then biomass production and thinning intervals for

plantations and forests can be optimized.

The size–growth relationship is one approach to analyze the growth partitioning among trees in stands, depending on their individual sizes. A large number of papers have been written on the size–growth relationship and its applicability to estimate the mode of competition (Hara, 1993). Starting in the 1980s, studies on the size–growth relationship have included nonwoody plants (e.g., Weiner and Thomas, 1986; Weiner, 1990; Gerry and Wilson, 1995; Weiner et al., 1997; Schwinning, 1996; Schwinning and Weiner, 1998), juvenile woody stands (e.g., Hara, 1993; van Kuijk et al., 2008), and mature forest stands (e.g., Stoll et al., 1994; Wichmann, 2001; Pretzsch and Biber,

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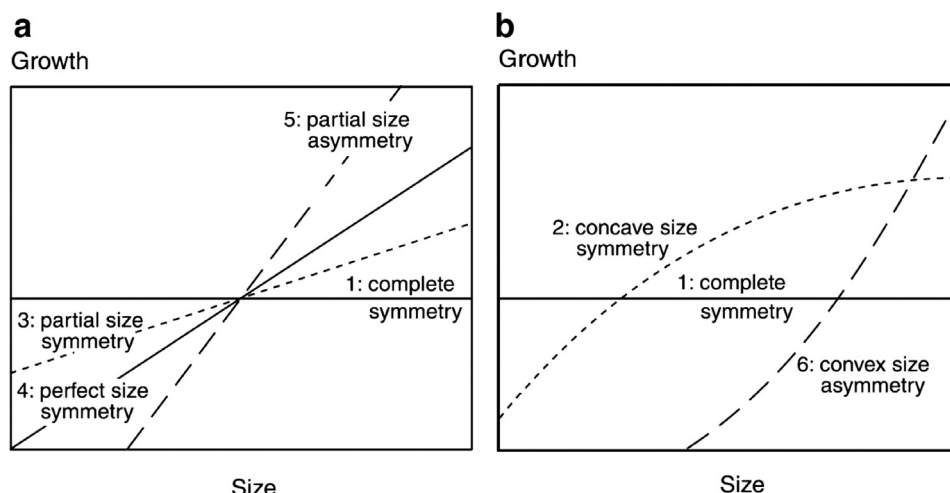
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increases (progressively) as size increases (Pretzsch et al., 2012).

2010; Pretzsch and Dieler, 2011; Dieler and Pretzsch, 2013, Forrester et al., 2013). In Fig. 1, different slopes of the size–growth relationship are presented, varying from size symmetry to asymmetry (competition), with intermediate forms (Pretzsch et al., 2012).

The slope of the size–growth relationship is mainly influenced by the supply and limitation of above- and below-ground resources (Weiner and Thomas, 1986; Weiner, 1990). An increasing slope of the size–growth relationship indicates an increasing availability of the below-ground resources and consequently increasing competition for above-ground resources, primarily sunlight (Weiner, 1990), so that competition becomes more size asymmetric (Fig. 1, Lines 5 and 6). Sunlight and resource partitioning is disproportionately available to larger trees, which shade smaller trees (Ford, 1975; Cannell and Grace, 1993; Weiner and Thomas, 1986; Schwinning and Weiner, 1998). In contrast, a decreasing slope indicates increasing competition for below-ground resources, primarily nutrients and water (Weiner, 1990; Krannitz et al., 1991; Schwinning and Weiner, 1998). The decreasing slope of the size–growth relationship is going to be more symmetric (Fig. 1, Lines 1, 2, 3, and 4). Irrespective of size, trees receive nearly the same resources and growth partitioning (Pretzsch et al., 2012). Consequently, depending on the resource supply and limitation, the slope of the size–growth relationship will increase or decrease. Thereby, the competition for sunlight depends on the availability of below-ground resources (Weiner, 1990).

Almost all European tree species are reliant on essential below-ground nutrients and cannot overcome a resource limitation. Nevertheless, *R. pseudoacacia* (family *Fabaceae*) is one example that is able to compensate for limited available soil nitrogen. With the symbiotic aid of rhizobia, black locust is capable of fixing nitrogen from the atmosphere (Hoffmann, 1961, 1964; Veste and Kriebitzsch, 2013). This ability appears to be an advantage when colonizing areas with low soil nutrients, especially nitrogen. Furthermore, *R. pseudoacacia* is a fast-growing, sunlight-loving, and relatively drought-tolerant tree species (Xu et al., 2009; Veste and Kriebitzsch 2013; Mantovani et al., 2014a, 2014b; Carl et al., 2017a, 2017b). Hence, this species has an increasing relevance for water- and nutrient-poor sites, such as post-mining sites in Germany (Grünwald et al., 2009; Carl et al. 2017a, 2017b) and the semi-arid Loess Plateau in China (Lei et al., 2016). However, detailed information about nutrition allocation and growth partitioning in *R. pseudoacacia* is not available.

The statistical approach to verifying the relevance of below- and above-ground resources to the mode of competition of *R. pseudoacacia* is the size–growth relationship (Fig. 1) in a mixed-effects model with a random intercept and slope, first, including relevant below- and above-ground resources and their interactions as fixed effects, and second,

Fig. 1. Depiction of the size–growth relationship between tree size and absolute growth rate, depending on above- and below-ground resources. (a) Different linear and (b) nonlinear relationships between size and growth (Pretzsch and Biber, 2010; Pretzsch and Dieler, 2011; Pretzsch et al., 2012). Line 1 represents completely symmetric size–growth relationship in which all trees received the same resources and growth partitioning irrespective of size (more theoretical case). Line 2 reflects nonlinear concave size symmetry where growth increases less (degressively) as size increases. Line 3 displays partial size symmetry in which growth increases linearly with size. Line 4 stands for perfect size symmetry, where growth increases proportionally with size. Line 5 represents partial size asymmetry where growth increases disproportionately with size. Line 6 displays nonlinear convex size asymmetry as the growth in-

quantifying the influence of further site-specific effects of the analyzed stands as random effects. Hence, this study deals with the following research question:

does the supply of nitrogen, phosphorus, and water as below-ground resources impact the competition mode of *R. pseudoacacia* by varying growth partitioning?

2. Methodology

2.1. Study area

In southern Brandenburg in North-East Germany (Fig. 2), 10 study sites, with a total of 27 sample plots and 850 (1333, including the neighboring boundary trees) sample trees (Table 1), were prepared and included in the study.

The sample area was characterized by an average temperature of 9.4 °C and an annual precipitation of 555 mm (1965–2015, from the nearest weather station in Cottbus (DWD, 2017)).

2.2. Soil data

Soil analysis was carried out to a depth between 0 and 1 m. The mean weighted average values (Table 2) are with reference to five soil depth horizon levels: humus layer, 0–10 cm, 10–30 cm, 30–60 cm, and 60–100 cm. For each individual soil horizon per site, selected nutrient concentration (mg kg^{-1}) and soil texture were determined, and the mean weighted average values were calculated (Table 2). The analytical methods were based on DIN EN ISO/IEC 17,025 (DIN, 2005). In the study areas, four soil types were recognizable on the basis of plasticity and grain size analysis (Köhn, 1928): sandy loam, strong loamy sand, weak loamy sand, and sand (Table 2). The available water capacity for the root system in the soil (AWC_r) (Benzler, 1982) ranged from 54 (–) to 170 mm (+), with the average value being 109 mm (–). The plant-available phosphorus concentration (P) was analyzed via calcium-acetate-lactate (CAL) extraction (Schüller, 1969) and photometry. P ranged from 7.8 (–) to 24.0 mg kg^{-1} (+), with an average value of 15.2 mg kg^{-1} (–). Mineral nitrogen concentration (N_{min}), consisting of nitrate (NO_3^-) and ammonium (NH_4^+), was analyzed via photometry (DIN, 2005) and ranged from 1.4 to 5.3 mg kg^{-1} .

2.3. Yield and competition data

At each of the 10 study sites, three sample plots were selected at random, with a minimum of 30 trees and a maximum of 39 trees per

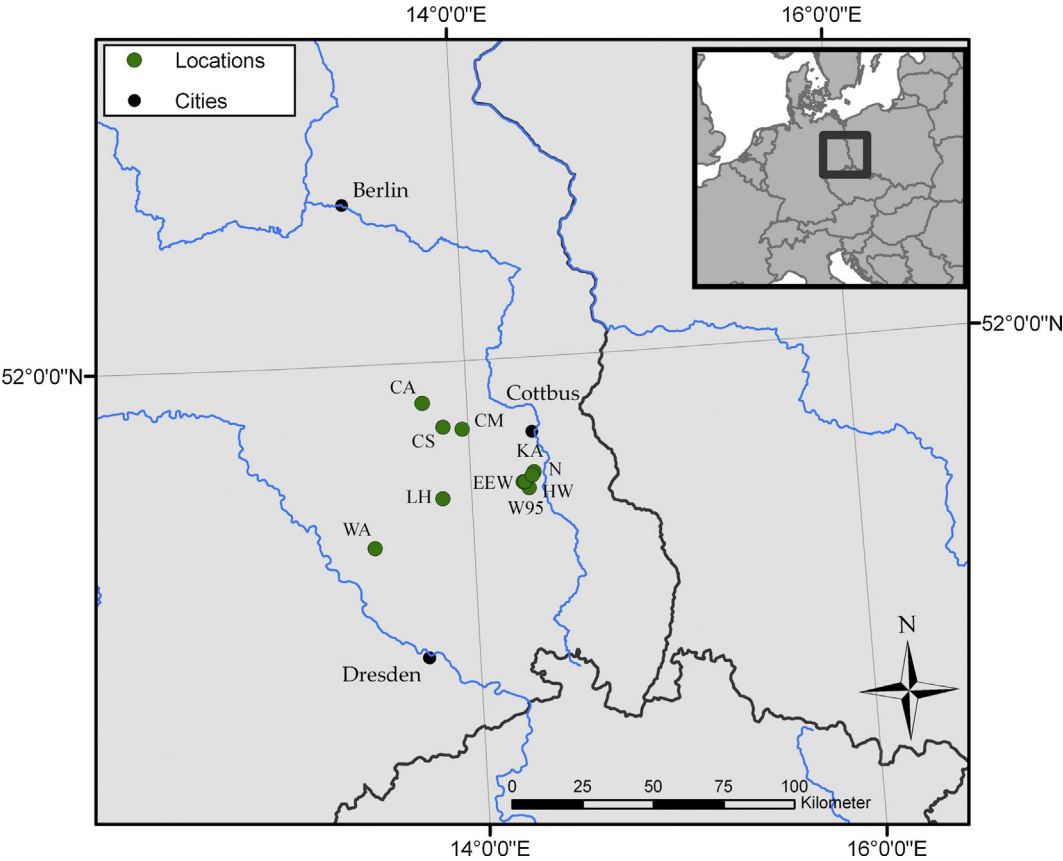


Fig. 2. Location of the 10 study stands of *Robinia pseudoacacia* L. included in this study. Abbreviations are explained in Table 1.

plot. Moreover, the boundary trees adjacent to sample plots were also surveyed. The field variables determined at each study site (Table 3) were the coordinates, diameter at breast height (DBH) ranging from 0.6 to 29.1 cm, height, height to crown base, and crown radii in the four cardinal directions. To calculate the growth per year, the DBH was measured in spring (20–24 April 2017) before leaf proliferation and in autumn (30–31 October 2017), at the end of the annual growth period. The basal area (BA) and the BA growth (gBA) were calculated with the DBH in a circumference function. The BA ranged from 0.3 to 6651 cm² and the gBA from 0 to 38.1 cm² a^{−1}.

The sunlight competition index (CI_{Light}) was calculated by applying the light cone method (Pretzsch et al., 2002), which is described by Pretzsch and Biber (2010). This method considered only the larger neighboring trees that reached into the cone. Therefore, Pretzsch and Biber (2010) assumed that most of a tree's relevant competitors are located within a radius of 2.5 times the tree's average crown width,

which was estimated from tree diameter, and height (Pretzsch et al., 2002). Furthermore, the tops of the competitors for sunlight will be contained within an inverted cone with an open angle of 60° originating from two-thirds of the central tree's height (Pretzsch and Biber, 2010). This is a very common concept for defining competitors, especially with respect to sunlight in growth modeling (Biging and Dobbertin, 1995; Pretzsch, 2009; Pretzsch and Biber, 2010). Furthermore, the sunlight-competitor trees were counted, the mean diameter (presented as the mean basal area per tree) was determined, and a modified local version of Stand Density Index (SDI: Reineke, 1933; Pretzsch and Biber, 2005) was calculated as sunlight competition index (CI_{Light}), according to Pretzsch and Biber (2010). The described CI_{Light} has the advantage of being invariant in relation to the development stage of a stand. The highest value of sunlight-competition index was CI_{Light} 1054 and the lowest value was CI_{Light} 8 (Table 3).

Table 1
Study site descriptions including number of sample plots, number of sample trees, location, longitude (Long), latitude (Lat), elevation above sea level (ASL), former utilization, trees per hectare, and tree age.

Location	Sample plots	Sample trees	Long (°E)	Lat (°N)	ASL (m)	Former utilization	Tree age (years)	Plants per hectare
Calau Mlade (CM)	3	93	13°58'12	51°46'52	93	Forest	32	1896
Calau restricted area (CS)	2	60	13°51'57	51°47'15	93	Forest	22	2464
Welzow train (W95)	3	99	14°17'55	51°34'43	123	Mining	22	2365
Welzow sewage plant (KA)	3	98	14°16'50	51°36'06	123	Mining	12	2415
Welzow Hühnerwasser (HW)	3	100	14°16'00	51°36'09	123	Mining	9	2946
Lauchhammer (LH)	3	96	13°51'14	51°33'19	111	Mining	8	4549
Welzow Nelder (N)	1	32	14°19'19	51°37'15	123	Mining	7	3966
Cahnsdorf (CA)	3	90	13°45'57	51°52'18	63	Agriculture	5	5361
Welzow EEW (EEW)	3	91	14°19'42	51°37'30	123	Mining	2	35,203
Wainsdorf (WA)	3	91	16°29'39	51°24'49	92	Agriculture	2	22,346

E = East, N = North () Abbreviation of the location.

Table 2

Soil data of the 10 study sites, mean weighted average values of soil measurements at 0–1 m. Locations: Welzow Train (W95), Welzow Hühnerwasser (HW), Welzow EEW (EEW), Welzow Nelder (N), Lauchhammer (LH), Welzow sewage plant (KA), Calau Mlode (CM), Calau restricted area (CS), Wainsdorf (WA), and Cahnsdorf (CA). Soil types (Köhn, 1928): sandy loam (SL), weak loamy sand (WLS), strong loamy sand (SLS), sand (S). AWC_r = available water capacity (Benzler, 1982), N_{min} = mineral nitrogen (photometry (DIN, 2005)), S_{min} = mineral sulfur (calcium chloride extraction, DIN, 2005), P = phosphorus (Schüller, 1969), K = potassium (Schüller, 1969), and Mg = magnesium (calcium chloride extraction (DIN, 2005)). Acronyms of the locations are explained in Table 1.

Location	Soil types	Water capacity	AWC_r (mm)	Water content (%)	pH-value	N_{min} (mg kg ⁻¹)	S_{min} (mg kg ⁻¹)	P (mg kg ⁻¹)	K (mg kg ⁻¹)	Mg (mg kg ⁻¹)
CM	S	Low	54	7.7	4.3	4.0	2.3	18.0	11.5	9.1
CS	S	Low	54	7.5	6.5	3.4	0.8	8.8	17.0	23.1
W95	SL	High	170	10.2	7.4	5.3	3.6	10.0	105.0	58.6
KA	WLS	Medium	136	5.8	6.9	3.5	1.4	9.0	35.0	34.5
HW	WLS	Medium	136	7.1	6.2	3.3	0.6	17.5	31.5	41.2
LH	SLS	High	142	16.4	3.5	1.7	11.6	7.8	26.5	371.6
N	WLS	Medium	128	4.7	7.3	2.4	2.2	9.3	27.8	67.7
CA	S	Low	54	4.6	5.8	3.3	0.7	24.0	44.0	35.7
EEW	WLS	Medium	91	8.4	6.9	1.4	26.1	24.0	26.5	30.6
WA	WLS	Medium	128	8.4	6.1	4.6	0.7	23.5	60.0	98.8

Table 3

Yield, growth, and sunlight-competition index data at the 10 study sites. Locations: Welzow train (W95), Welzow Hühnerwasser (HW), Welzow EEW (EEW), Welzow Nelder (N), Lauchhammer (LH), Welzow Sewage plant (KA), Calau Mlode (CM), Calau restricted area (CS), Wainsdorf (WA), and Cahnsdorf (CA). DBH = diameter at breast height, BA = basal area, CI_{Light} = sunlight-competition index. Values: mean (minimum and maximum). Abbreviations of the location are explained in Table 1.

Location	DBH (cm)	BA (cm ²)	BA growth (cm ²)	Height (m)	Crown base (m)	Crown radius (m)	CI_{Light}
CM	13.4 (4.2–29.1)	173.4 (13.8–665.1)	6.6 (0–35.2)	14.0 (3.3–23.5)	9.3 (1.9–20.0)	1.3 (0.3–3.4)	467 (74–1054)
CS	9.0 (3.6–17.3)	73.6 (10.2–234.5)	2.97 (0–19.2)	8.9 (3.1–15.2)	5.7 (1.9–10.2)	1.2 (0.5–2.5)	248 (56–487)
W95	10.3 (3.2–20.8)	100.1 (8.0–339.8)	6.8 (0–35.8)	10.9 (3.2–16.9)	6.5 (2.0–11.8)	1.5 (0.3–6.4)	338 (114–766)
KA	9.2 (4.5–16.4)	72.4 (15.9–211.2)	5.1 (0–29.1)	10.4 (5.4–14.1)	7.1 (3.1–10.5)	1.0 (0.2–2.1)	267 (74–526)
HW	8.3 (3.4–15.4)	60.8 (9.1–186.3)	6.9 (0–38.1)	9.7 (4.5–14.8)	5.9 (2.5–10.2)	1.3 (0.2–3.2)	289 (85–502)
LH	5.7 (1.7–12.5)	28.9 (2.3–122.7)	4.4 (0–20.4)	6.8 (2.4–9.4)	3.6 (1.5–6.4)	1.1 (0.3–1.7)	202 (17–564)
N	5.0 (2.5–8.0)	20.8 (4.9–50.3)	5.0 (0–19.8)	6.2 (3.2–7.5)	3.4 (2.0–4.7)	0.8 (0.3–1.4)	116 (29–231)
CA	4.5 (2.1–7.8)	17.0 (3.5–47.8)	5.0 (0–23.1)	5.9 (2.2–7.5)	3.5 (2.0–4.9)	0.8 (0.2–1.9)	167 (29–425)
EEW	1.72 (0.7–3.5)	2.7 (0.4–9.6)	1.3 (0–9.3)	2.6 (0.9–4.6)	0.9 (0–2.8)	0.2 (0–0.65)	214 (19–806)
WA	1.9 (0.6–3.9)	3.3 (0.3–12.0)	3.4 (0–14.4)	2.4 (1.0–3.9)	0.8 (0–2.2)	0.2 (0–0.7)	106 (8–259)
All sites	7.0 (0.6–29.1)	57.4 (0.3–665.1)	4.8 (0–38.1)	7.9 (0.9–23.5)	4.8 (0–20.0)	1.0 (0–6.4)	251 (8–1054)

2.4. Size–growth relationship

The size–growth relationship shows the growth partitioning among trees in a stand, depending on their size. This is one approach to analyze the competition for above- and below-ground resources. Using this approach, the mode of competition (Hara, 1993), via size–growth relationship of mature forest stands, was successfully applied by e.g. Stoll et al. (1994), Wichmann (2001), Pretzsch and Biber (2010) and Pretzsch and Dieler (2011). Accordingly, in Fig. 3, the size–growth relationship (using DBH as the measure of size and growth) of each of the 10 *R. pseudoacacia* stands at the study sites was plotted. The points were the observed tree data, and the lines are site-specific fitted linear regressions. The former agricultural areas (Fig. 3, blue) were characterized by a small DBH range and a high typical slope. The former mining areas (Fig. 3, red) was the sample group with the highest number of sites in the analyzed study and exhibited a typical slope intermediate between those from the former agricultural (high typical slope) and forest areas (low typical slope). Forests (Fig. 3, green) reveal the largest range of DBH values and a flattened slope. The size–growth relationship of the various study sites (Fig. 3) indicated a site-specific growth partitioning, with varying intercepts and slopes.

2.5. Statistical approach

The size–growth relationship (Section 2.4) was described in a mixed-effects model with a random intercept and slope.

First, model selection was carried out using the top-down strategy described by Diggle et al. (2002). Therefore, all explanatory variables (Section 2.2 and 2.3), as fixed components, were modeled and selected. The explanatory variables were chosen to be CI_{Light} (as above-ground resource) and, as below-ground resources P as the nutrient and AWC_r as

the water. Variables N , S , K , and Mg were omitted, as they had no influence to growth partitioning. The central part of Eq. (1) was the interaction between above- and below-ground resources to answer the research question; “might there be an impact of below-ground resources on the mode of competition?”. Thereof, second, as further explanatory variables, the interactions of $CI_{Light} * P$ and $CI_{Light} * AWC_r$ were integrated. Thus, depending on the resource supply and limitation, the slope of the size–growth relationship might be steeper or shallower. Accordingly, increasing key below-ground resources could be result in an increasing competition for sunlight, classified as asymmetric competition. Symmetric competition would be based on competition for limited below-ground resources. Consequently, if there was a significant ($p < 0.05$) interaction among below- (P , AWC_r) and above-ground (CI_{Light}) resources, then the mode of competition as growth partitioning depended significantly on the supply of phosphorus and water.

Third, the best size–growth relationship model, including all relevant explanatory variables to answer the research question, was selected. We decided to use the BA and gBA in a natural logarithmic transformation, caused by their high homoscedasticity compared with DBH and DBH growth. Moreover, as the response variable, we used gBA_{ij} as the gBA of tree j on site i and the corresponding explanatory variables of tree j on site i . We had 10 study sites; the trees at these 10 sites were likely to be more closely related to each other (Fig. 3) than to the trees from different sites. A linear regression model would not take this relatedness into account (Zuur et al., 2009). Hence, we preferred a mixed-effects model with a random intercept and slope. Mixed-effects models are characterized by two components that contain explanatory variables: fixed and random term, also described as fixed effects or overall components and random, subject or site-specific effects (Zuur et al., 2009). Moreover, fixed effects included population effects

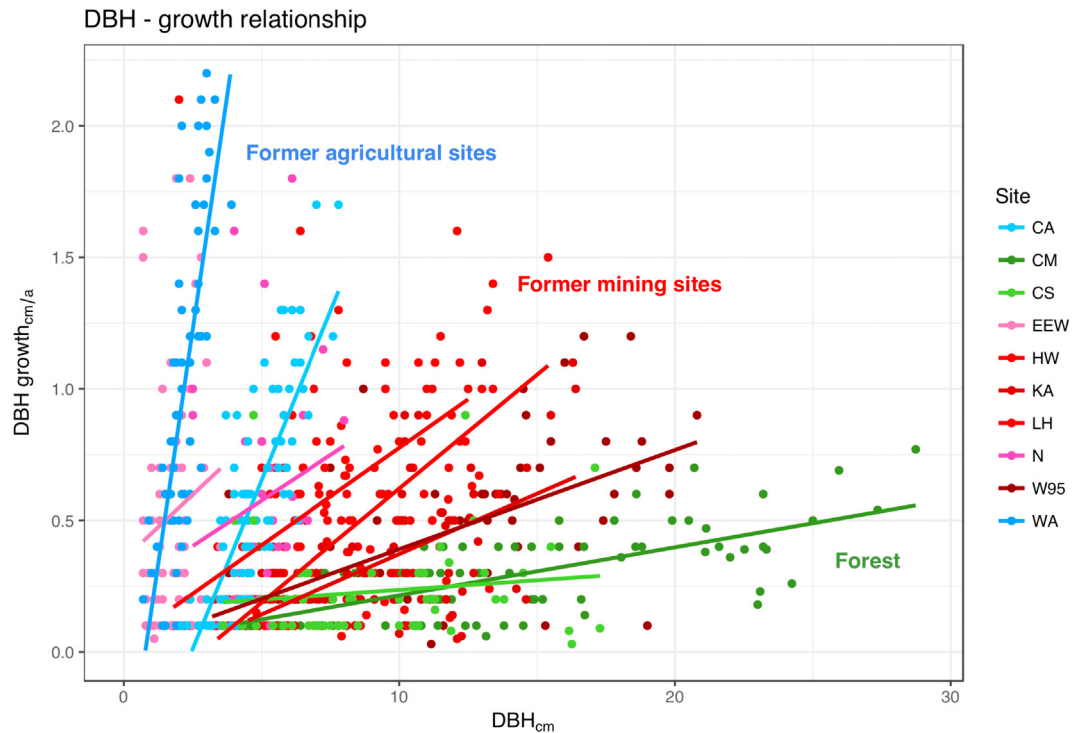


Fig. 3. Size–growth relationship (DBH) *R. pseudoacacia* at all 10 study sites, colored by the former utilization: blue = former agricultural areas, red = former mining areas, and green = forests. The colored points are the observed individual tree data per site. The colored lines are site-specific fitted linear regressions. Abbreviations for individual study sites are explained in Table 1. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(Fahrmeir et al., 2009). The fixed term in Eq. (1) contained all explanatory variables with the coefficients b_0 – b_6 . For the random term, we decided to use the random intercept c_i per site i and the random slope as $d_i \ln(BA_{ij})$ per site i . Thereby, random effects showed the possible influence of further unknown site-specific growth effects independent of the chosen explanatory variables. Moreover, ε_{ij} represented the independent observation error.

$$\ln(gBA_{ij}) = b_0 + b_1 \ln(BA_{ij}) + b_2 (CI_{Lightij}) + b_3 (P_{ij}) + b_4 (AWC_{rij}) + b_5 (CI_{Light} * P)_{ij} + b_6 (CI_{Light} * AWC_r)_{ij} + c_i + d_i \ln(BA_{ij}) + \varepsilon_{ij} \quad (1)$$

All in all, the statistical approach shows the influence of the chosen explanatory variables on growth partitioning, depending on their availability. For all statistical and graphical support in this study, we used the software packages R, version R 3.3.2 (R Core Team, 2016b), application packages “stats” (R Core Team, 2016a), “nlme” (Pinheiro et al., 2017), “ape” (Paradis et al., 2004), and “ggplot2” (Wickham, 2009).

3. Results

The results from the estimation of the fixed and random effects of the mixed-effects model (Eq. (1)) are shown in Table 4.

Starting with the fixed term, the estimates of the coefficients (b_1 , b_2 , b_3 , and b_4) show that, with increasing values for BA , CI_{Light} , P , and AWC_r , the response variable gBA also increased. Thereby, the explanatory variables BA and P had the strongest influence. Furthermore, increasing values for the interactions of $CI_{Light} * P$ and $CI_{Light} * AWC_r$ ($-b_5$, $-b_6$) were associated with a decrease in the gBA values. The estimates of the coefficients $-b_5$ and $-b_6$ had the strongest effects on trees on fertile sites (high P and AWC_r values) with a high CI_{Light} value, primarily small trees with many light competitors. Consequently, with increasing site quality as indicated by the key below-ground resources,

P and AWC_r , the slope of the size–growth relationship increased and became more asymmetric (Fig. 4). Hence, the greatest slope was on the most fertile sites, because the growth partitioning of trees with many light competitors (i.e., small trees) exhibited the greatest reduction in the interaction among above- and below-ground resources. In contrast, large trees on fertile sites benefited from the greater influences of the estimates for coefficients (b_1 , b_3 , and b_4) of BA , P , and AWC_r as well as the slight reduction as a result of the interactions of $CI_{Light} * P$ and $CI_{Light} * AWC_r$, caused by low CI_{Light} values. In contrast, on sites with increasing limitation of P and AWC_r , the influence of the explanatory variables P and AWC_r was much slighter than on fertile sites, and the slopes of the size–growth relationship was more symmetric and flattened (Fig. 4).

The results of the estimates of the random term are presented in the last three columns of Table 4. There were considerably higher variances of the intercepts (0.746) than of the slopes (0.065) at the 10 study sites. The correlation (corr) between the intercept and slope of the random term was -0.655 . This indicated that sites with a high positive slope generally had a high negative intercept and vice versa (Fig. 3). Where there was a strong site-specific effect of further unknown variables, then the significance of the fixed effects would be lost by inserting random effects. Nevertheless, the estimates of the $CI_{Light} * P$ and $CI_{Light} * AWC_r$ interactions remained significant ($p < 0.05$) when including the random intercept and slope. Thereof, our results were independent of further random and unknown site-specific effects. Ceteris paribus, the supply or limitation of phosphorus and water as the key below-ground resources affected the slope and intercept of the size–growth relationship as well as the competition mode, by varying growth partitioning significantly.

In Fig. 4, the size–growth relationship results from Table 4 were plotted with defined varying values of the explanatory variables. The high (+), average (~), and low (–) values for the variables used in Fig. 4 are based on the measured and calculated maximum, mean, and minimum values (Sections 2.2 and 2.3). High P (+) and AWC_r (+)

Table 4
Explanatory variables and the coefficient estimates of Eq. (1). Fixed effects are on the left side and random effects on the right side (separated by the broken line). BA = basal area, CI_{Light} = sunlight competition index, P = Phosphorus (mg kg^{-1}), AWC_r = available water capacity of the roots, SE = standard error of the estimate, StdDev = standard deviation, and corr = correlation. Significance level: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, ns not significant.

Variable	Coefficient	Estimate	SE	p-value	Significance	Variance	Std Dev	Corr
$\ln(BA)$	b_0	−4.7138	1.1272	< 0.0001	***	0.74594	0.86368	
	b_1	0.9933	0.0961	< 0.0001	***	0.06507	0.25510	−0.655
CI_{Light}	b_2	0.0028	0.0015	0.0616	ns			
P	b_3	0.1074	0.0410	0.0397	*			
AWC_r	b_4	0.0122	0.0063	0.1007	ns			
$CI_{Light} * P$	b_5	−0.0001	0.0001	0.0461	*			
$CI_{Light} * AWC_r$	b_6	−0.00002	< 0.0001	0.0306	*			

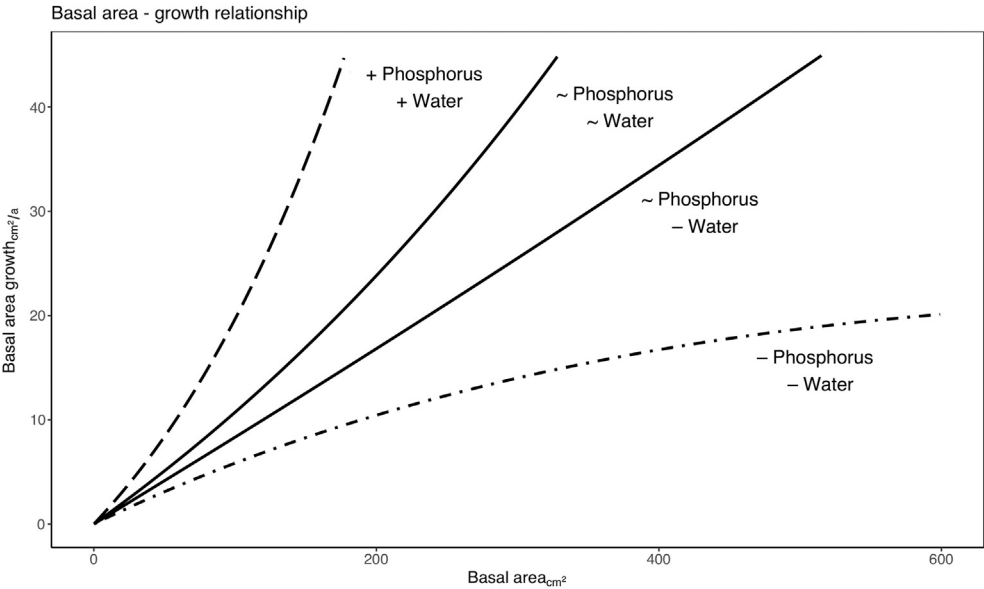


Fig. 4. Basal area–basal area growth relationship. The long dashed line represents high phosphorus and water availability (+) and is convex size asymmetric, whereas the solid line with average phosphorus and water availability (~) is convex size symmetric. The solid line with average phosphorus values (~) and a low water availability (−) is size symmetric, while the dot-dash line with low phosphorus and water availability (−) is concave size symmetric.

values showed that taller trees benefit and have disproportionately high growth partitioning then do smaller trees. As a result, in the BA–gBA relationship, the long dashed line was convex size asymmetric. Average P (~) and AWC_r (~) values resulted in convex size symmetric competition, whereas average P (~) and low AWC_r (−) values resulted in size symmetric competition. Hence, the competitiveness of trees increases proportionately with the tree size. Low P (−) and AWC_r (−) values displayed concave size symmetry. Additionally, taller trees received minor growth partitioning compared with stands with high P and AWC_r values. Hence, the competition for below-ground resources increased with decreasing P and AWC_r availability.

4. Discussion

Does the supply of nitrogen, phosphorus, and water as below-ground resources impact the competition mode of *R. pseudoacacia* by varying growth partitioning? No and yes. First, nitrogen as a below-ground resource had no impact on the competition mode. As we pointed out, that *R. pseudoacacia*, being a member of the *Fabaceae* family, is capable of fixing atmospheric nitrogen with the aid of different rhizobia (Hoffmann, 1961,1964; Veste and Kriebitzsch, 2013) and is able to compensate for limited available soil nitrogen. Second, phosphorus and water as below-ground resources are the key drivers of the growth partitioning. Ceteris paribus, the supply of phosphorus and water influenced the slope and intercept of the size–growth relationship. Increasing phosphorus and water availability brought about increasing competition for above-ground resources, primarily sunlight (Fig. 4). That phosphorus is the most important nutrient for *R. pseudoacacia* growth is in line with the findings of Kanzler et al. (2015).

They concluded that phosphorus fertilization increased the growth of *R. pseudoacacia* seedlings (Kanzler et al., 2015). Furthermore, compared with other plant families, members of the *Fabaceae* require more phosphorus for their root development, and energy-driven processes that are central to maintenance of their mutualistic relationship with nitrogen-fixing rhizobia (Olivera et al., 2004; Hernandez et al., 2009; Rotaru and Sinclair, 2009; Cabeza et al., 2014). Therefore, it seems that plant-available phosphorus improves the phosphorus and nitrogen cycles of the *Fabaceae* (Olivera et al., 2004; Hernandez et al., 2009; Rotaru and Sinclair, 2009; Cabeza et al., 2014) appreciably, for example, in *R. pseudoacacia* trees, by increasing growth and competition. This effect could play the important key role in the growth partitioning and competition mode of *R. pseudoacacia*. Nitrogen is generally classified as an important below-ground resource (nutrient) for other species (Weiner, 1990; Wichmann, 2001; Pretzsch and Biber, 2010) but can also be classified as an above-ground resource (in terms of atmospheric nitrogen) for *R. pseudoacacia*, depending on the symbiotic activity of nitrogen-fixing rhizobia capable of fixing nitrogen gas from the air. On the contrary, the factors controlling phosphorus flux have been little understood until recently (Bol et al., 2016; Lang et al., 2016), but the influence of phosphorus on processes of growth partitioning, for example to wood, leaf, or cell, could be investigated in further studies.

After phosphorus, water availability is the second most important below-ground parameter, influencing the growth partitioning, the effects probably being intensified by the dryness of the sandy study areas. Our result, that high water availability generates increasing size asymmetric competition, is in line with the findings of Schwinning and Weiner (1998) and Wichmann (2001). Moreover, if water availability is limited, smaller plants and trees may be able to keep their stomata open

for longer (Barnes et al., 1990), as a result of their position in the mid-canopy, where the evaporative demand is lower. The relatively strong growth reduction in tall trees can be interpreted as an internal trade-off in tree metabolism between growth and defense mechanisms (Pretzsch et al., 2012).

R. pseudoacacia is a tree species planted and growing in dry and marginal areas, for example, former mining areas in Germany (Mantovani et al., 2013; Carl et al., 2017a, 2017b) and the semi-arid Loess Plateau in China (Lei et al., 2016). However, *R. pseudoacacia* is not a water-saving tree species (Mantovani et al., 2014a), but has developed different morphological and ecophysiological strategies for adaption to survive drought periods and to grow in dry spatial regions. Under these conditions, *R. pseudoacacia* responds with leaf inclination (Xu et al., 2009), leaf loss, renewable leaf production over the whole year (Veste and Kriebitzsch, 2013), and increasing nodule biomass (Mantovani et al., 2015). All of these adaptive strategies suggest that the relative growth rate of *R. pseudoacacia* during dry periods might be reduced.

Why is the statistical approach we used applicable to analysis of the competition mode? First, the size–growth relationship showed that growth partitioning among trees in a stand depended on their size. A number of papers have studied the size–growth relationship and its use to estimate the competition mode (e.g., Weiner and Thomas, 1986; Weiner 1990; Hara, 1993; Weiner et al., 1997; Schwinning, 1996; Schwinning and Weiner, 1998; Wichmann, 2001; Pretzsch and Biber, 2010; Pretzsch and Dieler, 2011). They concluded, that the slopes of the size–growth relationship curves indicate different possibilities of symmetric and asymmetric competition. In this study, depending on the supply of phosphorus and water, the slope of the size–growth relationship increased or decreased. Second, inclusion of the interactions sunlight * phosphorus ($CI_{Light} * P$) and sunlight * water ($CI_{Light} * AWC_r$) enabled us to analyze explicitly how growth partitioning depended on the supply of phosphorus and water as well as the interaction between selected below- and above-ground resources. This approach was the main part of the study to answer the research question. The significant influence of the interactions showed that there was a strong correlation between phosphorus and water limitation to the competition for sunlight. Third, mixed-effects models have the advantage that fixed and random effects are included. Thereby, fixed effects present the population effects and random effects show the possible influence of site-specific unknown effects. In our study, by including the random intercept and slope, the results stayed significant. As a consequence, there was no overlaying influence of unknown site-specific effects, such as, for example, the stand development, age growth trend, etc. That meant that our results on key drivers of the competition mode and growth partitioning, phosphorus and water, were statistically significant, with high probabilities. Nevertheless, we point out that our study involved primarily stands with a diameter range from 0.6 to 29.5 cm in areas with sandy soils in Germany. The translation of these conclusions to different site conditions and diameter classes may be possible, given the independence of the model to site conditions.

What does this research mean for forest ecosystem management and forest practice? The competition as well as the optimal tree density for high biomass production differed depending on water and phosphorus availability. In line with Pretzsch et al. (2012), we concluded that the size–growth relationship reflected the resource supply and affected the structural diversity in a stand. On poor sites, shallow slopes indicated adequate growth conditions for small trees, while steep slopes indicated self-thinning of the understory trees as their tall neighbors obtained more sunlight and growing space. On rich sites, a forest manager can support a limited number of high-quality trees. Therefore, thinning operations can be concentrated on undesirable and small trees in the stands, because understory trees are difficult to maintain. On poor sites, the thinning intervals can be protracted, because the competition and the self-thinning processes are rather slower. Further studies could quantify the optimal plant density and thinning intervals of *R.*

pseudoacacia per site, based on the availability of the limiting below-ground resources, water and phosphorus. Moreover, the approach of including site-specific soil data and following the statistical approach of the current study could be extended to include different tree species, stands, mixed forest stands, and sites.

5. Conclusion

The key drivers of the competition mode of *R. pseudoacacia*, by their effect on growth partitioning, were phosphorus and water. With increasing supply of phosphorus and water, the slope of the size–growth relationship and competition for sunlight increased. On the contrary, increasing limitation of phosphorus and water was associated with decreasing slope of the size–growth relationship and decreasing competition for sunlight, as well as the increasing competition for below-ground resources. Plant-available nitrogen as below-ground resource had no influence on the competition mode. The results from our research came from stands with mean diameters ranging from 0.6 to 29.1 cm in areas with sandy soils in Germany. Nevertheless, the statistical analysis, using a mixed-effects model with a random intercept and slope, showed that our results are independent of further site-specific random effects and would probably be transferable to additional sites with different characteristics. Overall, the competition for sunlight increased and the optimal tree density for a high biomass production decreased with increasing water and phosphorus availability. Consequently, practical operators could, in the future, adapt their tree density to the site-available resource conditions to optimize the biomass production of *R. pseudoacacia* plantations and forest stands.

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Author contributions

Hans Pretzsch, Dirk Landgraf, Maik Veste, Peter Biber, and Christin Carl initiated and planned the project; Christin Carl performed the experiments, analyzed and modeled the data with Peter Biber, and wrote the manuscript. Dirk Landgraf, Maik Veste, Peter Biber, and Hans Pretzsch contributed by revising the manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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Article 3: Flower Analysis - Honey Potential

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Carl et al. 2017b

Article

Robinia pseudoacacia L. Flower Analyzed by Using Unmanned Aerial Vehicle (UAV)

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Abstract: Tree flowers are important for flower–insect relationships, seeds, fruits, and honey production. Flowers are difficult to analyze, particularly in complex ecosystems such as forests. However, unmanned aerial vehicles (UAVs) enable detailed analyses with high spatial resolution, and avoid destruction of sensitive ecosystems. In this study, we hypothesize that UAVs can be used to estimate the number of existing flowers, the quantity of nectar, and habitat potential for honeybees (*Apis mellifera*). To test this idea, in 2017 we combined UAV image analysis with manual counting and weighing of the flowers of eight-year-old black locust (*Robinia pseudoacacia* L.) trees to calculate the number of flowers, their surface area, and their volume. Estimates of flower surface area ranged from 2.97 to 0.03% as the flying altitude above the crowns increased from 2.6 m to 92.6 m. Second, for the horizontal analysis, a 133 m² flower area at a one-hectare black locust plantation was monitored in 2017 by a UAV. Flower numbers ranged from 1913 to 15,559 per tree with an average surface area of 1.92 cm² and average volume of 5.96 cm³. The UAV monitored 11% of the total surface and 3% of the total volume. Consequently, at the one-hectare black locust study area we estimate 5.3 million flowers (69 kg honey), which is sufficient for one bee hive to survive for one year.

Keywords: unmanned aerial vehicles (UAVs); tree blossoms; *Robinia pseudoacacia* L.; honeybees

1. Introduction

Flower production is essential for the survival of most plant species in the world, because it relates directly to the number of seeds produced. As a side effect, flowers provide ecosystem services, such as food for many insects via the flower–pollinator relationship. Moreover, humans have benefited for thousands of years; for example, by cultivating honeybees (*Apis mellifera*) for honey production or for the pollination of fruit trees. Furthermore, not only are tree flowers, but also the subsequent fruits and seeds, energy banks for many animals. However, detailed information about tree flowers, particularly those of black locust (*Robinia pseudoacacia* L.), is very limited.

Black locust is one of the most criticized non-native tree species in Germany and Europe [1] because its rootstocks spread into neighboring areas. Furthermore, black locust represses rare, native, and endangered species [2]. However, black locust also offers many advantages and thus may represent an opportunity for European forests in these times of climate change. For example, it is a fast growing, sun light loving, and unpretentious tree and can grow in poor-nutrient areas, such as former brown-coal mining areas. In addition, black locust starts flowering early, often at an age of

six years [3] or younger. Many insects, especially *Hymenoptera*, such as *A. mellifera*, benefit from this service. Black locust honey is often sold as acacia honey [4], and in Hungary, the production of this type of honey is very common and economically important [3].

Many ecosystem structures and changes may be monitored via satellite data [5–10]. In forest ecosystems, the focus has been on the canopy structure [11–13], especially the leaf area index (LAI) [13–17], chlorophyll analysis [13,18,19], and discoloration caused by diseases or disturbance [20–22], particularly insects [23–31]. In Africa, flower cycles have been monitored by airplane remote sensing [32,33] as a guide for beekeepers. However, unmanned aerial vehicles (UAVs) allow monitoring with a much higher spatial resolution of small, specific, and detailed vegetation structures, such as flowers. In addition, UAVs combine quick turnaround times in combination with lower operational costs. Therefore, UAV data may provide an important supplement to data from satellites, airplanes, towers [34], and to field-collected data. In agriculture, the work done with information and communication technology is described as the fourth revolution. Smart farming is the key to developing sustainable agriculture, and UAVs are an important part of this strategy [35]. Scientific and practical examples of UAV use include the monitoring of agave crops [36], canola [37,38], maize [39,40], potato [41,42], wheat [42–47], sugarcane [48], viticulture [49,50], peach trees [51], olive trees [52–54], and papaya trees [55] in addition to the distribution of biological insecticides [56] and the control of drainage systems in agricultural areas [57]. UAVs are also a part of a new revolution in forestry management [58]. For example, UAVs are now used to find bark beetles as early as possible [59,60], to monitor the infestation levels of pests [61], to manage wild animals, develop forest inventories [58,62], monitor wind damage [63], schedule projects, exploit forests [64], and to quantify the expected seed production of beech trees [65]. In plant ecology, UAVs have been used for LAI analysis [66] and to analyze the structure of ultrafine dryland vegetation [67]. Furthermore, Mc Neil et al. [68] combined a UAV and ImageJ (a freeware Java-based image-processing program) to measure leaf-angle distribution in the canopies of broadleaved trees. MacInnis and Forrest [69] used ImageJ to quantify the pollen deposition of two *Narcissus* ssp. and strawberry (*Fragaria x ananassa*). Flower-health classification supported by digital-imaging techniques with ImageJ is the topic of Lino et al. [70].

In the present study, we use a UAV and flower analysis by ImageJ as an innovative and economical option to analyze the flowers of *R. pseudoacacia* and estimate the food base for *Hymenoptera*, especially *A. mellifera*. We investigate 13 different flying altitudes (Vertical Analysis), monitor a $50 \times 50 \text{ m}^2$ area (Horizontal Analysis), and seven sample trees (Flower Trees) in bloom in an eight-year-old black locust plantation. The flowers were counted, photographed, and weighed. Furthermore, we create two models: Model (1) solely base on the field-collected data, and Model (2), which combines the field-collected data with photographic data from the UAV.

The main idea of this study is to monitor the insect-biodiversity potential of black locust trees by indirect analysis. To demonstrate this method, we apply it to honeybees, although numerous opportunities exist for analyzing further insect species. Thus, this study represents a first step toward developing a method to analyze tree flowers, which may eventually evolve into an indirect method to analyze biodiversity. The research objectives of this study are (1) to adapt methods to classify flowers in black locust plantations via UAV red–green–blue (RGB) aerial imagery; and (2) to evaluate the number of flowers and the nectar production, and to assess the habitat for honeybees. The corresponding research questions are as follows:

Can UAVs be used to analyze flowers in *R. pseudoacacia* plantations?

- (1) What is the best flying altitude (FA) for the UAVs?
- (2) How much flower surface (2D) and volume (3D) can be detected by a UAV?
- (3) How many flowers are present per hectare, and per tree?

Is this method suitable to monitor the structure of the temporary habitat for honeybees?

- (1) What mass of nectar and honey (kg) can be produced by one hectare of eight-year-old *R. pseudoacacia*?

- (2) What population of honeybees can survive for one year from the flower production of a one-hectare black locust plantation?

2. Materials and Methods

2.1. Study Area

The study area is in northeastern Germany in the federal state of Brandenburg (13°50'57''E, 51°32'20''N, and 111 m above sea level). It has an annual precipitation of 555 mm and a mean annual temperature of 9.4 °C [71]. The study area was previously used for brown-coal mining and has now been recultivated with different tree species [72]. The black locust plantation under study was planted eight years ago. Blossoms flower at the end of May and at the beginning of June (29 May–2 June 2017). The analysis began with the flight of a hexacopter [56] on 29 May 2017.

2.2. Flower Analysis

The analysis was divided into three parts (Figure 1). First, a Vertical Analysis was done to determine differences in the detected study area and the best flying altitude. This was followed by a Horizontal Analysis to get an overview of the entire area. Finally, the Flower Trees were intensively analyzed to estimate the number of flowers and nectar for honeybees.

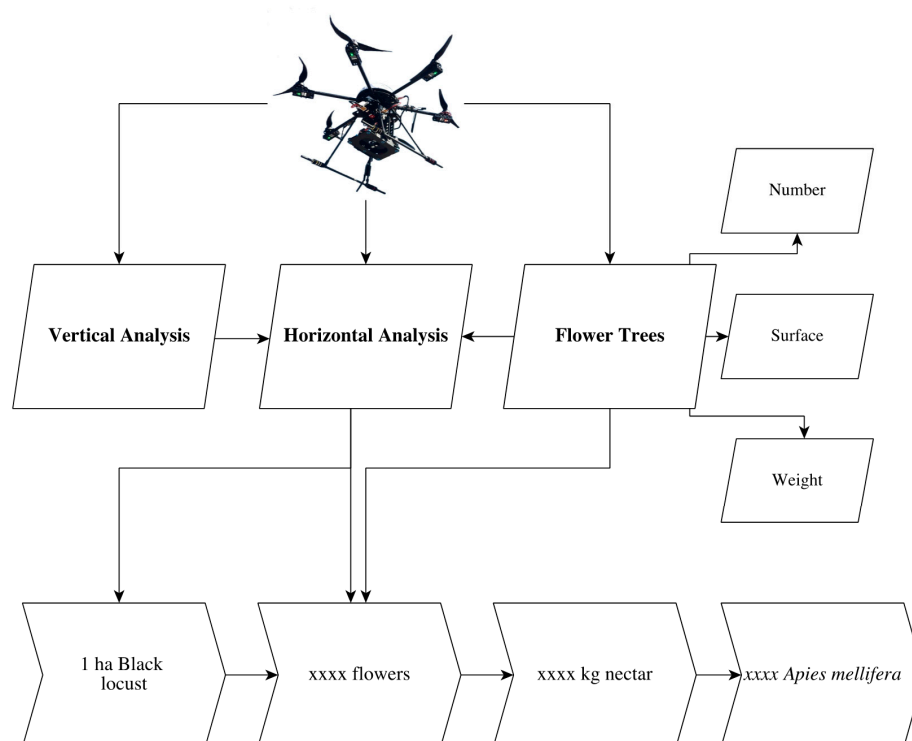


Figure 1. Flow chart showing analysis procedure (Vertical, Horizontal Analysis, and Flower Trees) to estimate flowers per tree and hectare, mass of nectar in kg, and number of *Apies mellifera* [56,73].

2.2.1. Vertical Analysis

For the Vertical Analysis we chose 13 different flying altitudes (FA) above the starting point: 20 to 50 m in 5 m steps, and 50 to 110 m in 10 m steps (Table 1). The FA above the starting point was determined by the UAV on-board computer. The FA above ground and above crown was calculated (Appendix A). A FA of 20 m above the starting point was chosen because it was the lowest FA the UAV could fly without significant buffeting of the canopy from rotor downwash. In total, 26 pictures

were taken by the on-board MAPIR Survey2 RGB camera (MAPIR, San Diego, CA, USA) [74] for the Vertical Analysis.

Table 1. Vertical Analysis: Flying altitude (FA) of hexacopter above starting point, ground, and crowns.

FA Above Starting Point (m)	FA Above Ground (m)	FA Above Crowns (m)
20.0	8.2	2.6
25.0	13.2	7.6
30.0	18.2	12.6
35.0	23.2	17.6
40.0	28.2	22.6
45.0	33.2	27.6
50.0	38.2	32.6
60.0	48.2	42.6
70.0	58.2	52.6
80.0	68.2	62.6
90.0	78.2	72.6
100.0	88.2	82.6
110.0	98.2	92.6

2.2.2. Horizontal Analysis

For the Horizontal Analysis, the hexacopter flew at a low FA (2.6–5.5 m above crowns, Table 2) above a $50 \times 50 \text{ m}^2$ square area. Therefore, 80 areas were delineated within a grid with a cell size of 6.2 m (8 x lines) by 5.0 m (10 y lines). Scale bars were positioned on the ground. The black border of each scale bar is $50 \times 50 \text{ cm}^2$ and the white panel is $25 \times 25 \text{ cm}^2$ (Figure 2). The Field of View (FOV) is based on sensor size (6.17472 mm $\times$ 4.63104 mm), focal distance (3.97 mm), and height above ground (m) (Table 2). The Ground Sample Distance (GSD) is calculated from the FOV and pixel values (4608 $\times$ 3456). Images are individually cropped to prevent the photographs from overlapping. All in all, 160 photographs (two photographs of each subarea) were created with a MAPIR Survey2 RGB camera (MAPIR, San Diego, CA, USA) [74] for the Horizontal Analysis. The FA is influenced by the slope of the area (Appendix A), and in each line the flying altitude above the starting point, ground, and crown varies (Table 2).

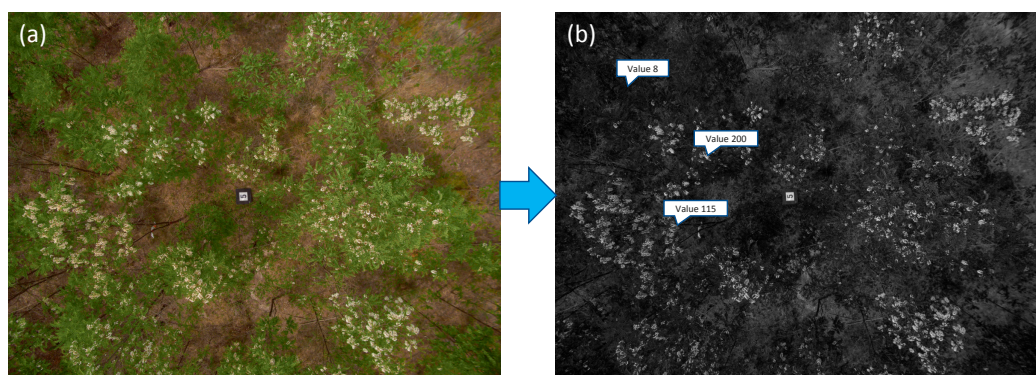


Figure 2. Red–green–blue (RGB) aerial picture (a) with the scale bar in the middle of the field. Blue-channel view of picture (b) with three example pixel values.

Table 2. Horizontal Analysis: Flying Altitude (FA) of the hexacopter above starting point, ground, and crowns and the Field of View (FOV) above the ground.

Flight Line (South-North)	FA Above Starting Point (m)	FA Above Ground (m)	FA Above Crowns (m)	FOV (Above Ground) (m)	(m)
A	17.0	10.8	5.5	16.8	12.6
B	18.0	10.5	5.3	16.3	12.3
C	19.0	10.0	4.5	15.6	11.7
D	19.0	8.5	3.0	13.2	9.9
E	20.0	8.2	2.6	12.8	9.6
F	22.0	9.1	4.4	14.2	10.6
G	24.0	10.0	4.5	15.6	11.7
H	24.0	9.2	4.2	14.3	10.7
Mean	20.4	9.5	4.2	14.9	11.1

2.2.3. Flower Trees (UAV and Field Data Collection)

Seven Flower Trees were used to compare and supplement the UAV data with field-collected data. The scale bars were installed in the crowns of the Flower Trees, and the UAV flew to each Flower Tree separately. The flower area of each Flower Tree individually was calculated by using ImageJ (Section 2.2.4). The reference data consists of the manually analyzed flowers per tree. The Flower Trees are the base to estimate the relationship between flowers detected by UAV and the total number of manually counted flowers, the total flower surface, and the total flower volume. This relationship is used to calculate the number of flowers for the analyzed $50 \times 50 \text{ m}^2$ area and per hectare. For the field data collection, the seven marked Flower Trees were harvested shortly after the UAV flights (30 May–2 June 2017). Furthermore, each racemose inflorescence of each branch was analyzed, and the total number of flowers was counted. Next, 50 inflorescences were selected at random and the exact surface area of each flower was determined for 10 flowers from each inflorescence (500 flowers total). This was done by photographing each flower and using ImageJ [75,76] to determine the surface area. The binary function of ImageJ is helpful for measuring the surface area per flower. To calculate the flower volume (i.e., the 3-dimension flower surface (3D)), the flowers were weighed (digital precision scale $\pm 0.001 \text{ g}$, maximum 20 g). Therefore, we determine the total mass of each flower and the mass of a 1.0 cm^2 area of each flower.

The relationship between the number of flowers and the percent of flower surface detected by the UAV is determined via a linear regression analysis and the coefficient of determination.

2.2.4. Image Analysis

ImageJ is used for image analysis. ImageJ is a free, Java-based image-processing program and Fiji is an open-source image-processing package that operates in the ImageJ software environment [75,76]. After converting the image to red, green, and blue channel, we used the blue channel for flower analyses. The advantage of the blue channel is that the tree biomass (leaves, weeds, branches) reflect poorly in the blue band. Therefore, a distinct boundary appears between flowers and tree (and weed) biomass (Figure 2).

The flower area was calculated after defining the threshold value for all three subdivisions: Vertical (threshold 110–160), Horizontal (threshold 140), and Flower Tree Analysis (threshold 140). By analyzing 12,500 pixels (ImageJ segmentation versus user/visual inspection of flowers), threshold 140 had the best Overall Accuracy (99.5%) and Kappa Coefficient (0.913) (Appendix A, Table A1). Threshold 120–150 had an almost perfect (0.81–1.0) level of agreement to the reference and threshold 110 and 160 were classified as substantial (0.61–0.81) by using the Kappa Coefficient [77,78]. Thresholds smaller than 140 had a low Users Accuracy for the Flower classification (Commission Error). Therefore, parts of the biomass and soil are classified as flowers. Increasing thresholds (>140) have a decreasing Producers Accuracy for the Flower classification (Omission Error). Consequently, flowers are classified as biomass. Hence, threshold 140 was used for Horizontal Analysis and the Flower Trees. The topology

of the study area is rough; the angle of slope averaged 12° . Therefore, the FA at the starting point differs from the FA above the ground and crowns over the entire area (Vertical Analyses, Table 3), above Flower Trees, and in the Horizontal Analyses in each line (Figure 2, Table 2). Details of the computed FAs, scale bars, and the crown-correction factor appear in Appendix A, especially Figure A1 and Equations (A1)–(A7).

2.2.5. Models: Calculating Flower Number

We developed two models to calculate the number of flowers. First, by using only field-collected data, we calculate the flower number $n_{\text{flowers}}^{(1)}$ by multiplying the average flower number $\bar{n}_{\text{flowers}}$ of the Flower Trees and the counted number n_{trees} of trees per hectare. Explicitly, Model (1) is calculated as follows:

$$n_{\text{flowers}}^{(1)} = \bar{n}_{\text{flowers}} n_{\text{trees}} \quad (1)$$

In the second model, the flower number $n_{\text{flowers}}^{(2)}$ is calculated based on the field-collected data combined with the UAV data. The flower area of one hectare (FoA) is determined by using the average percent yielded by the horizontal analysis of $\frac{1}{4}$ hectare and the average flower surface area FS_U detected by the UAV. Explicitly, Model (2) is calculated as follows:

$$n_{\text{flowers}}^{(2)} = \frac{FoA}{FS_U} \quad (2)$$

All analyses were done by using R, version R 3.3.2 [79], with the R packages “stats” [79], “ape” [80], “ggplot2” [81], “png” [82], and “gridGraphics” [83].

2.2.6. Nectar, Honey, and Honeybees

We use information from the literature [2,84–87] to estimate the nectar, honey production, and food base for *A. mellifera* in the study area. *R. pseudoacacia* blossoms flower for 5.5 days [2]. Black locust produces 1.7–3.7 mg nectar per flower over 24 h, and the nectar has a sugar concentration of 34–67%, so the sugar mass per flower over 24 h is 0.9–2.3 mg, and the honey production reaches 159–1000 kg per hectare [84]. On average, one bee hive requires 70 kg honey per year. In the summer (winter), 30–50,000 (10–15,000) honeybees live in each hive [85–87].

Additionally, one week after the flowering period for black locust, we collected the honey of two beekeepers with hives close to the study area. The honey analysis is done in the laboratory (University Hohenheim) and with a refractometer (Brix scale (sugar concentration): 58–90% with a precision 0.5%, Baumé (Bé) 38–43° with 0.5° Bé precision, 12–27% water with 1% precision). Water and sugar concentration and the pollen of both nectar-giving plants and nectarless plants are analyzed in each honey sample.

3. Results

3.1. Vertical Analysis

Figure 3 shows a bird’s-eye view from various FA above the crowns (2.6–92.6 m) with different variables in the blue channel (threshold 110–160). The image shows a decreasing flower surface with increasing FA. The relative flower surface ranges from 2.965% at threshold 110 (shaded and illuminated flowers, some parts of soil) at 2.6 m FA above the crowns to 0.027% threshold 160 (illuminated flowers) at 92.6 m FA above the crowns. The detected area for all FA greater than 42.6 m above crowns covers for each threshold 110–160 similar values such as FA 42.6 m. The strongest signal of decreasing detected flower area occurs at 12.6 m FA above crowns.

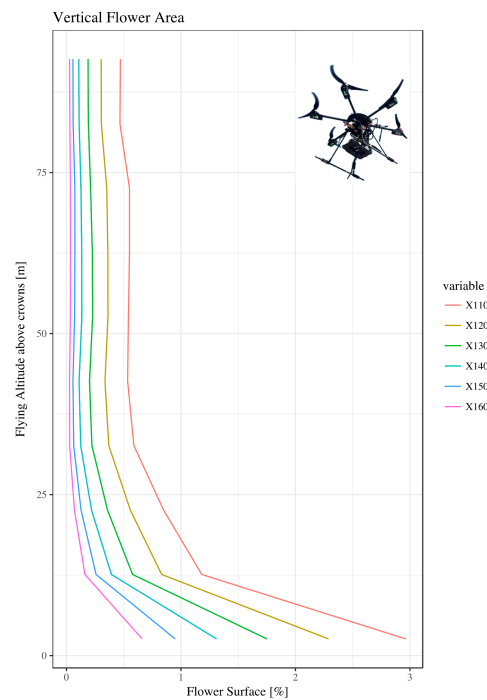


Figure 3. Vertical flower surface (%) for the Flying Altitude (FA) of 2.6–92.6 m above crowns. Graphs are based on different thresholds (X110–X160) in the blue channel.

3.2. Horizontal Analysis

The analyzed $50 \times 50 \text{ m}^2$ area is summarized by dividing it into 40 by 80 subareas (Figure 4). Each subarea is represented by a $6.0 \times 10.4 \text{ m}^2$ grid. The average detected flower area covered 1.33% (threshold 140) out of the total area under study. The minimum (maximum) detected flower area is 0.212% (2.612%). The highest (lowest) flower concentration occurs in field E1 (H1). Figure 4 shows that fewer flowers are present in the southeastern part of the area.

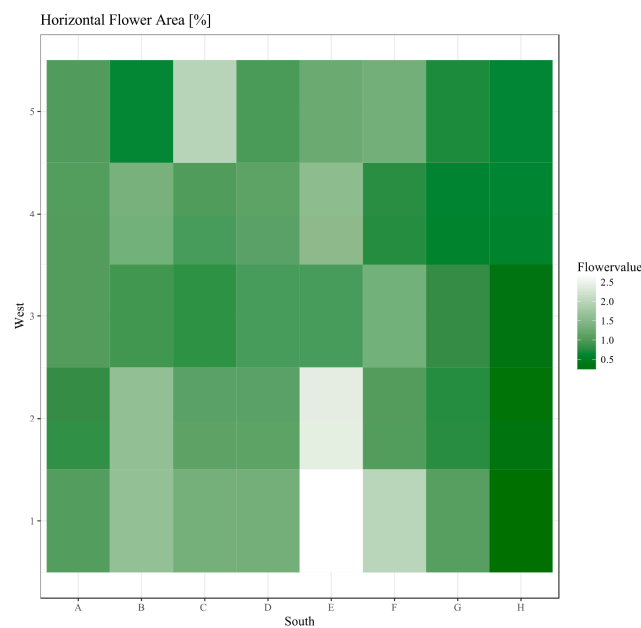


Figure 4. Horizontal flower surface in percent. Maximum is 3.0% (white) and minimum is 0.1% (dark green) for the analyzed fields in the $50 \times 50 \text{ m}^2$ area by using threshold 140.

3.3. Flower Trees, Flower Surface, and Volume (2- and 3-Dimensional)

The flower area per Flower Tree detected by the UAV ranges from 242 cm² to 16,468 cm² by using threshold 140. A manually count of the number of inflorescences per tree (Table 3) ranges from 84 to 658. On average, 20 flowers are counted per inflorescence. Flowers per tree range from 1913 to 15,559. Tree number two, which was one of the biggest trees in the stand, had a huge number of flowers. Tree two is the highest Flower Tree at 7.2 m (the average tree height was 5.7 m), has the biggest circular crown area of 3.1 m² (average circular crown area is 1.8 m²) and diameter-at-breast height of 6.0 cm (average diameter-at-breast height is 4.8 cm). Therefore, a significant correlation exists between the greatest tree-size parameters and the highest flower number. For clarity, tree number two is deleted in Figure 5b. The 2D flower surface has a minimum of 0.81 cm², a mean of 1.92 cm², and a maximum of 3.26 cm². The volume or 3D flower surface has a minimum of 3.29 cm³, a mean of 5.96 cm³, and a maximum of 12.19 cm³.

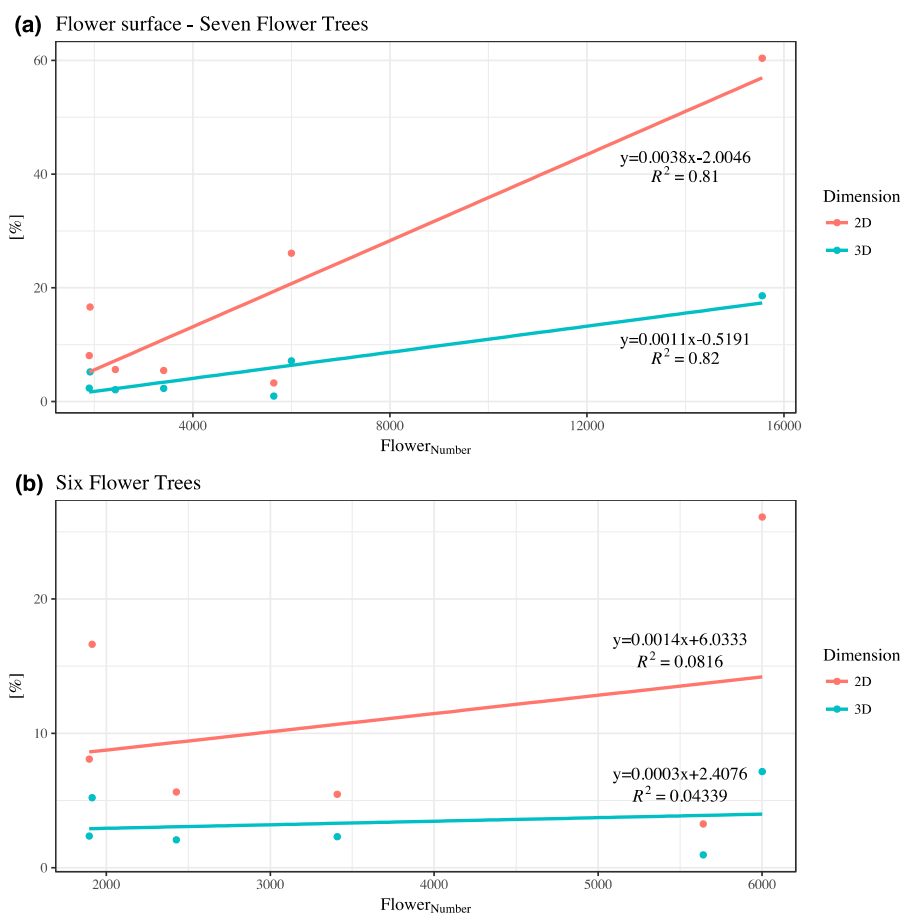


Figure 5. The 2D (red line) and 3D (blue line) flower surface of the (a) seven and (b) six Flower Trees. The percentage of flower surface detected by the unmanned aerial vehicle (UAV) is shown as a function of the number of flowers (Flower_{Number}). The linear regression functions and coefficients of determination appear in the legends.

Table 3. Overview of analyzed Flower Trees and the characteristics of their flowers; counted inflorescence, and flowers per tree, flower per inflorescence, flower surface, flower weight in total, 1.0 cm², and flower volume in cm³.

Tree Number	Inflorescence per Tree	Flower per Inflorescence	Flower per Tree	Flower Surface 2D (cm ²)	Flower Mass (Total)	Flower Mass (1.0 cm ²)	Flower Volume 3D (cm ³)
1	197	17 (1–34)	3409	2.44 (1.64–3.26)	133 (93–168)	24 (14–35)	5.78 (4.27–9.26)
2	658	24 (1–41)	15,559	1.75 (0.81–2.49)	100 (60–142)	18 (11–25)	5.69 (3.77–8.13)
3	324	19 (2–30)	6001	1.84 (1.21–2.57)	119 (82–215)	18 (10–24)	6.73 (4.66–11.46)
4	198	12 (1–25)	2427	1.90 (1.32–2.59)	90 (60–127)	18 (10–31)	5.15 (3.29–8.53)
5	204	28 (6–37)	5641	1.82 (1.29–2.26)	115 (77–226)	19 (8–27)	6.18 (4.31–12.19)
6	84	23 (2–38)	1913	2.12 (1.57–2.99)	105 (80–130)	16 (9–30)	6.77 (3.63–10.52)
7	118	16 (7–31)	1896	1.58 (1.22–2.10)	95 (72–123)	18 (11–25)	5.42 (3.98–7.82)
Mean	255	20 (3–34)	5264	1.92 (0.81–3.26)	108 (60–226)	19 (8–35)	5.96 (3.29–12.19)

Mean (minimum and maximum).

The 2D and 3D flower surfaces of the seven and six (without tree number two) Flower Trees are presented in Figure 5a,b, respectively. The standard error of the 2D flower surface is 7.7 by using all Flower Trees and 3.6 without tree number two. The standard error of the flower volume is 2.3 for all Flower Trees and 1.0 without tree number two. The coefficient of determination for both 2D and 3D measurements is much higher in Figure 5a then in Figure 5b. The detection method was relatively insensitive from 0 to 6000 flowers per tree. Based just on the seven sample trees, no significant correlation appears between flower number and flower surface as detected by the UAV. However, on average the UAV detected 10.86% of the total 2D flower surface and 3.34% of the total 3D flower surface. The average flower surface was 1.92 cm² (2D), of which 0.21 cm² were detected by the UAV. In addition, the mean flower volume was 5.96 cm³ (3D), of which 0.20 cm³ were detected by the UAV.

3.4. Flowers, Honey, Apies Mellifera

The two models used to calculate the number of flowers provide very different estimates. Model (1) estimates 11.9 million flowers per hectare and Model (2) uses the arithmetic mean of the horizontal area to estimate 5.3 million flowers per hectare. Using the minimum and maximum percent given by the Horizontal Analysis gives 1.0 to 12.5 million flowers per hectare. Model (1) does not consider flower failure. Model (2) may be used to calibrate Model (1), which overestimates by 56% the number of flowers. The food base for honeybees is calculated by using Model (2) and data from the literature [2,84–87]. Overall, 5.3 million flowers per hectare may provide 87 kg of nectar (69 kg of honey) and, if just *R. pseudoacacia* flower, may support a single bee hive for one year (Figure 6).

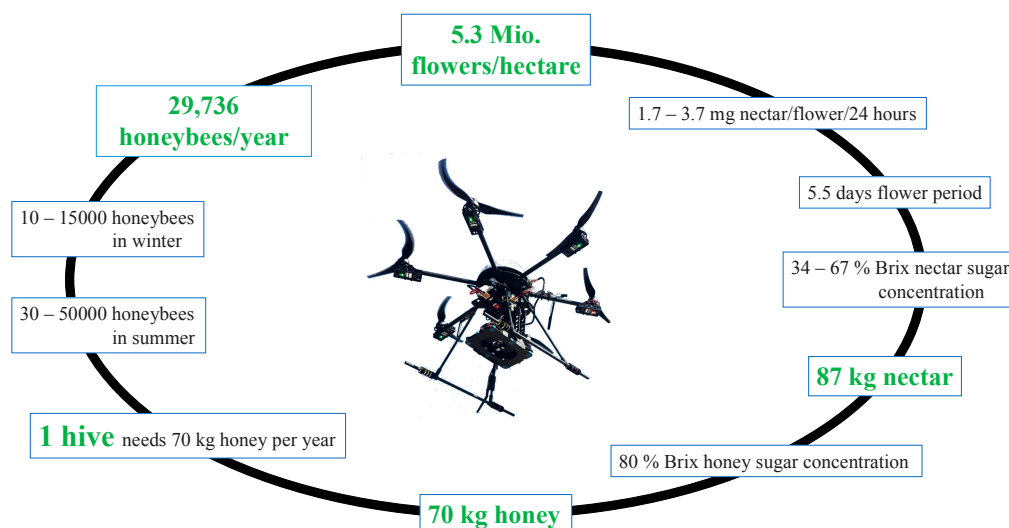


Figure 6. Flow chart connecting flowers, nectar, and honeybees per hectare. The green-text is the data calculated by using Model (2), the black text is data from the literature [2,84–87].

Honey analysis by refractometer shows 81% Brix honey sugar concentration and 16% water. In the laboratory, the microscopic investigation of pollen (DIN 10760 (a) (A)) [88] (535 and 551 pollen analyzed) reveals that the honey of both beekeepers can be considered *R. pseudoacacia* (acacia honey). The pollen of nectar-giving and nectarless plants is classified in Table 4. Overall, 19 different nectar-giving plants and 11 different nectarless plants were counted, in addition to *R. pseudoacacia*, by summing up all the data of honey samples 1 and 2.

Table 4. Microscopic pollen analysis of nectar-giving plants (first row) and nectarless plants (second row) from two honey samples (DIN 10760 (a) (A)) [88].

Plants	Classification	Honey Sample 1	Honey Sample 2
Nectar-giving	>45%	–	Brassicaceae
	45–15%	<i>R. pseudoacacia</i> , Brassicaceae	–
	<15–3%	Rubus, Salix, Cyanus segetum Hill	Pyrinae, <i>R. pseudoacacia</i> , Rubus, Salix
	<3%	Aesculus hippocastanum, Acer, Sinapis, Trifolium, Asteraceae, Pyrinae, Cornus, Gleditsia	Asteraceae, Aesculus hippocastanum, Liliaceae, Tilia, Weigela, Lytherum, Carduoideae, Frangula alnus
Nectarless	13%	Poaceae, Papaver, Asteraceae, Pyrinae, Cornus	–
	6.4%	–	Papaver, Quercus, Betula, Pinus, Sambucus, Filipendula ulmaria, Rosaceae

4. Discussion

The use of UAVs in agriculture [36–38,56,57,62], horticulture [51], plant ecology [66,68,89], and forestry [59,61,62,65] is increasing worldwide. However, UAVs were not heretofore used to quantify the flower production of black locust. In this work, by using a hexacopter and image analysis, we test a method to analyze the flowers of black locust trees in a short-rotation coppice on a previous brown-coal mining area in northeastern Germany. In addition, the flowers of seven sample trees were analyzed manually to calculate the number of flowers and to estimate the nectar and the honey production available as the food base for honeybees.

Based on the UAV and field-collected data, our model estimates 5.3 million flowers at the eight-year-old one-hectare black locust for the year 2017. *R. pseudoacacia* produces a minimum of 1896 flowers, a mean of 5264 flowers, and a maximum of 15,559 flowers (analysis of Flower Trees). Williams et al. [90] analyzed *Eucalyptus nitens* flowers in Tasmania and found a minimum of 8 flowers per tree and a maximum of 211 flowers per tree [90]. The flower production of *R. pseudoacacia* is high compared with that of *E. nitens*, but the flowers of *R. pseudoacacia* are smaller than those of *E. nitens* and the flower-insect relationships differ between the two tree species. Furthermore, *E. nitens* flowers for three months (from January to March) [91], whereas *R. pseudoacacia* flowers only 5.5 days [2]. Williams et al. [90] counted flowers manually. An additional analysis done with the aid of UAVs would have been possible because the white color of *E. nitens* flowers differs significantly from the colors of other elements, such as leaves, and branches, and rocks.

In the present study, 11% of the total 2D surface and 5.5% of the total volume (3D) of the *R. pseudoacacia* flower area are captured by the UAV. For future studies, we recommend analyzing more than seven flower trees to obtain a more accurate coefficient of determination (regression) and more significant correlations between flower number and detected flower area. Horton et al. [51] obtained an average detection success rate of 84.3% for peach flowers. However, peach plantations have the advantage that the trees flower before foliation. In addition, the stand density is lower, and the peach trees are smaller than black locust trees. Horton et al. [51] explains that some of the white and light-pink flowers are similar in color to the branches and some parts of the ground. In Germany, *R. pseudoacacia* normally flower at the end of May after foliation. Therefore, many flowers are concealed by leaves, branches, or other flowers.

Near-infrared cameras were useful for collecting debilitation of plants caused by nutrient deficiency (potassium) or insect attacks (green peach aphid or bark beetle) and for calculating the

normalized density vegetation index [37,59,61,92]. In the present study, the blue channel is used to analyze the flowers in the UAV pictures. This corresponds to the description of Horton et al. [51]: objects with high chlorophyll (weeds) reflect strongly in the near-infrared and green bands but poorly in the blue band. In our study, because of the chlorophyll, the leaves reflect strongly in the near-infrared and green bands. In the blue channel, the biomass (chlorophyll) reflects poorly, but the white flowers reflect strongly. Therefore, a noticeable boundary exists between flower and tree (and weed) biomass. Richardson et al. [34] used the relative brightness of the red channel (red%) to analyze flowers of red maple trees. Because the flowers of red maple trees are red, the best boundary is obtained by using the red channel. Overall, flower analysis is possible in most cases with a RGB camera. The use of the blue channel to analyze the flowers of black locust works well. We tested various thresholds in the blue channel (110–160). Shaded and illuminated flowers (and some parts of the soil) are detected at threshold 110, whereas only illuminated flowers are detected at threshold 160. Consequently, at threshold 160 the User Accuracy Flower and the Producer Accuracy Biomass were 100%. However, 46.6% from the flowers are detected as biomass and get lost for the calculations (Appendix A, Table A1). When comparing all thresholds, threshold 140 performed best with respect to Overall Accuracy and Kappa Coefficient [77,78] and was therefore used in Horizontal and Flower Tree analyses to measure the flower area.

The Vertical Analyses are similar to that applied by Severtson et al. [37] with the vertical FA ranging from 20 to 110 m above the starting point. The flower surface of the entire area (leaves, branches, stones, soil, etc.) ranges from 2.97% to 0.03% at FAs of 2.6 and 92.6 m above the crowns, respectively. This indicates that flowers occupy just a small fraction of the environment in black locust plantations, and that most flowers are detectable if the UAV flies as near as possible to the crowns. As of 12.6 m FA above crowns, the detected flower area decreases rapidly. The size of the flowers does not change, but the area monitored by the UAV increases. The GSD changes rapidly with increasing FA. Therefore, the white color of the flowers merges with the darker colors of the leaves, branches, weed, and soil. Consequently, the threshold per pixel decreases. The detected area remains essentially constant for all FAs greater than 50 m above the starting point. Radiometric calibration is not applied, because radiometric distortions, normally caused by atmospheric influences, were insignificant considering the very low FAs [61,93]. Furthermore, the detected area does not significantly increase if the flower number increases from 0 to 6000 flowers per tree: the number of flowers detected remains nearly constant, except for tree number 2 of 60% detected flower area. Tree number 2 had a large number of flowers (15,000 flowers per tree) compared with the other Flower Trees. Tree 2 was not in a different phenological state as the other trees. One explanation for the recognized large number of flowers and area is; tree 2 was one of the biggest (diameter, height, circular crown area) trees in the stand. Higher trees tree might benefit more from sun light for energy production, and the bigger circular crown area indicates a high number of branches that can produce more flowers altogether. The use of the crown-correction factor is advised if the ground-slope angle is greater than 10° , but it is also important if the tree height varies along and between the lines in the study area. Therefore, the crown-correction factor is one possible way to improve the precision of the results. Tree growth rate varies from tree to tree, which leads to variations in tree height, branches, and crown structure.

Model (2) of this study estimates 5.3 million flowers for one hectare of eight-year-old *R. pseudoacacia* plantation. As a result, one bee hive could survive one year from the estimated honey base. In the previous brown-coal mining area of Lauchhammer, 40 hectares were planted with *R. pseudoacacia* over the last 10 years. Moreover, older black locust forests and black locust in mixed stands are neighboring to the study area. Therefore, at least 40 *A. mellifera* hives could survive, if all replanted areas (40 hectare) have nearly the same number of flowers as the study area. In these times of loss of biodiversity [94–97], this study shows that re-planting of human dispelled landscape, such as former mining areas, can be beneficial to the food base, especially for honeybees. To understand the relationships between flower production and stand density, tree age, tree diameter, and tree height, further studies in different areas are recommended.

The estimated honey base of this study is 70 kg per hectare, which is the honey mass required per year for an average *A. mellifera* hive [85]. However, 70 kg per hectare is scant, compared with results from Hungary. In Hungary, nectar and honey yield of black locust is increases from year 6 to year 15. The maximum nectar yield is 836 kg/ha of nectar and 418 kg/ha of honey. The lowest yield is documented for year 36 with 384 kg/ha of nectar and 192 kg/ha of honey [3]. These results of Hungarian scientists are consistent with those of Droege [84], who reports that honey production of *R. pseudoacacia* ranges from 195 to 1000 kg/ha. Therefore, the 70 kg/ha calculated in this study is lower than the lowest limit of Droege [84] (195 kg/ha). This results may be attributed to the cold weather that occurred in mid-April 2017; with frost on 16 and 17 April 2017. In addition, the weather was not optimal during flower time. On 30 May, a thunderstorm destroyed some flowers. Surprisingly, beekeepers in Germany and the authors of this study observed more than one flowering period in 2017. In some areas in the south and the middle of Germany black locust flowered in mid-June and again at end of August. Further studies and observations are thus needed to get an overview of this behavior and to find mechanism behind it. However, the main flowering period is the end of May and one of the big challenges of flower analysis and honey production of black locust, especially for scientists, but also for non-local beekeepers, is the short time during of the flowering period, which lasts on average 5.5 days [2]. One week after the main flowering period of *R. pseudoacacia* our two beekeepers extracted the honey from their hives. The laboratory of the University of Hohenheim classified the honey as *R. pseudoacacia* honey. However, a microscopic pollen investigation revealed 30 different pollens of nectar-giving and nectarless plants in the two honey samples. These results show that, during the flowering time of *R. pseudoacacia*, a lot of other plant species produced energy spending flowers or pollen for honeybees. The focus of this study has been on a monoculture (plantation). However, biodiversity and ecosystem health benefits from the presence of many different plant species, and animal species as part of the biocoenosis of the ecosystem. Thus, the analysis of flowers is important to understand ecosystems worldwide.

Natural scientists, researcher, politicians, and layman are constantly learning more about the sensitivity of the environment from the results of UAV-based analyses. UAV technology is a sustainable technology that provides high-resolution analyses and minimal environmental damage. Therefore, the methodology of this study and of other studies [39,51,52,65] should be further tested to determine whether it is applicable to other tree species of differing ages and in mixed-forest stands. One hypothesis is that older trees probably have a different vertical crown structure (more branch layers) and thus contribute a different proportion of captured flowers. In mixed-forest stands, different tree species are part of the community and have differing flowering times during the year and different flowering-time durations. Flower analysis is difficult in complex ecosystems but provides an interesting indicator of the plant-animal (tree-insect) relationship, ecosystem health, and biodiversity.

5. Conclusions

This study proposes a methodology to analyze flowers in tree plantations and tested the method by applying it to an eight-year-old *R. pseudoacacia* plantation in a former mining area in Germany. The flower analysis was done by using images acquired by a UAV that were analyzed with the aid of the image analyzing software ImageJ. Seven Flower Trees were manually accessed to obtain reference data. Overall, most flowers are detectable if the UAV flies as near as possible to the crowns. The vertical flying analysis shows that the flower area is 2.97% (threshold 110), and 1.33% (threshold 140) at 2.6 m FA above the crowns, and 0.03% (threshold 160) at 92.6 m. The UAV captured 11% of the 2D flower surface and 3% of the flower volume (3D) of *R. pseudoacacia* flowers. Thus, the model estimates 5.3 million flowers are present in the analyzed one-hectare black locust plantation. On average, one honeybee hive could survive one year from the 2017 analyzed flowers of the one-hectare area of black locust.

The methodology and results presented herein provide local managers, foresters, and scientists with the opportunity to estimate flower productivity of black locust and further tree species worldwide.

This study demonstrates that RGB images acquired by UAVs can be used effectively to obtain rapid, economical, and meaningful estimates of flowers, nectar, honey production, and honeybee potential.

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Author Contributions: Hans Pretzsch, Dirk Landgraf and Christin Carl initiated and planned the project; Christin Carl performed the experiments, analyzed the data with Peter Biber, and wrote the manuscript. Dirk Landgraf, Peter Biber, Marieke van der Maaten-Theunissen and Hans Pretzsch, contributed by revising the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript:

2D	Two-Dimensional, flower surface area
3D	Three-Dimensional, flower volume
FA	Flying Altitude
FoA	Flower Area
FOV	Field of View
FSU	Average flower Surface detected by UAV
GSD	Ground Sample Distance
LAI	Leaf Area Index
RGB	Red-Green-Blue
UAV	Unmanned Aerial Vehicles

Appendix A

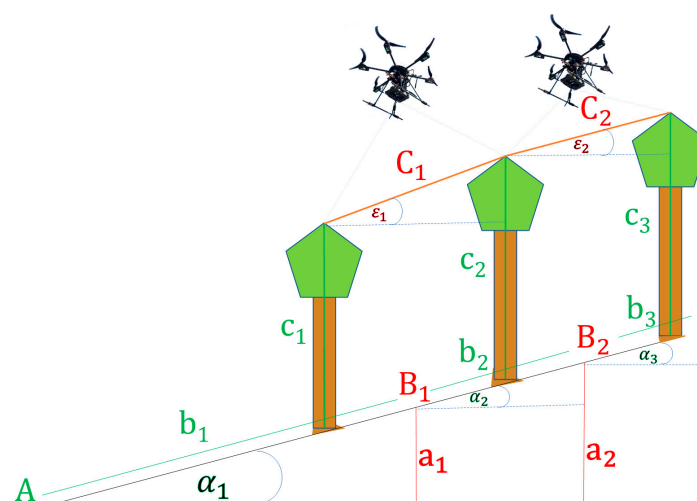


Figure A1. Real flying altitude above starting point measured by UAV on-board computer (A), above ground (B), and above crown (C): distance to rocks (a), slope angle (α), distance between the lines (b), tree height (c), angle and slope between two tree crowns (ϵ). All in-field measurements are in green and the calculated values are in red.

The real FA above ground (B) is calculated by using the FA from the starting point (A) minus the distance to rocks (a):

$$B = A - a \quad (\text{A1})$$

Therefore, a is calculated by using the slope angle (α) and distance between the lines (b) for each line analyzed (Figure A1):

$$a = b \sin(\alpha) \quad (\text{A2})$$

Scale bars were placed on the ground in the forest stand (Vertical and Horizontal Analysis). When calculating the flower area, the scale bar should be adapted to the crowns, where the flowers are located. Therefore, the FA above the crowns (C) is calculated from the FA above the ground (B) minus the tree heights (c):

$$C = B - c \quad (\text{A3})$$

The real size of the scale bars (d) is calculated by using the real FA above ground (B) and the viewing angle (β):

$$d = 2 * \sqrt{\left(\frac{B}{\cos\left(\frac{\beta}{2}\right)}\right)^2 - B^2} \quad (\text{A4})$$

To calculate the viewing angle (β) the FA above the crowns (C) and scale bar at the floor (e) are needed:

$$\beta = 2 \arctan\left(\frac{e/2}{C}\right) \quad (\text{A5})$$

Because the flowers are located in the crowns and the slopes of the crowns differ, a crown-correction factor (D) for the pictures is obtained by using the crown angles (ε):

$$D = \frac{1}{\cos(\varepsilon)} \quad (\text{A6})$$

Input data for the crown angle are the angle of the ground slope (α), the distance between trees (b), and the tree height (c) (Figure A1). Moreover, the difference in height between trees in the first line (c_1), and in the second line (c_2), and so on is calculated:

$$\varepsilon = \alpha + \arctan\left(\frac{c_2 - c_1}{b}\right) \quad (\text{A7})$$

Table A1. Accuracy assessment of flower detection by UAV in 3 m flying altitude above crowns. Therefore 12,500 pixels were analyzed (ImageJ segmentation versus user/visual inspection of flowers) and two classes were used: Flower and Biomass. In the blue channel for six different thresholds is calculated the Overall Accuracy, the Producer Accuracy (Omission Error), the Users Accuracy (Commission Error), the Mean Accuracy, and the Kappa Coefficient.

Threshold	Overall Accuracy (%)	Producers Accuracy (Flower)	Producers Accuracy (Biomass)	Users Accuracy (Flower)	Users Accuracy (Biomass)	Mean Accuracy (%)	Kappa Coefficient
>110	98.6	100	98.5	66.2	100	90.8	0.789
>120	99.1	99.4	99.1	75.5	99.9	93.4	0.854
>130	99.4	97.0	99.5	84.3	99.9	95.7	0.899
>140	99.5	89.2	99.8	94.0	99.7	98.2	0.913
>150	99.2	70.7	99.9	99.2	99.1	99.2	0.822
>160	98.7	53.4	100	100	98.7	99.0	0.690

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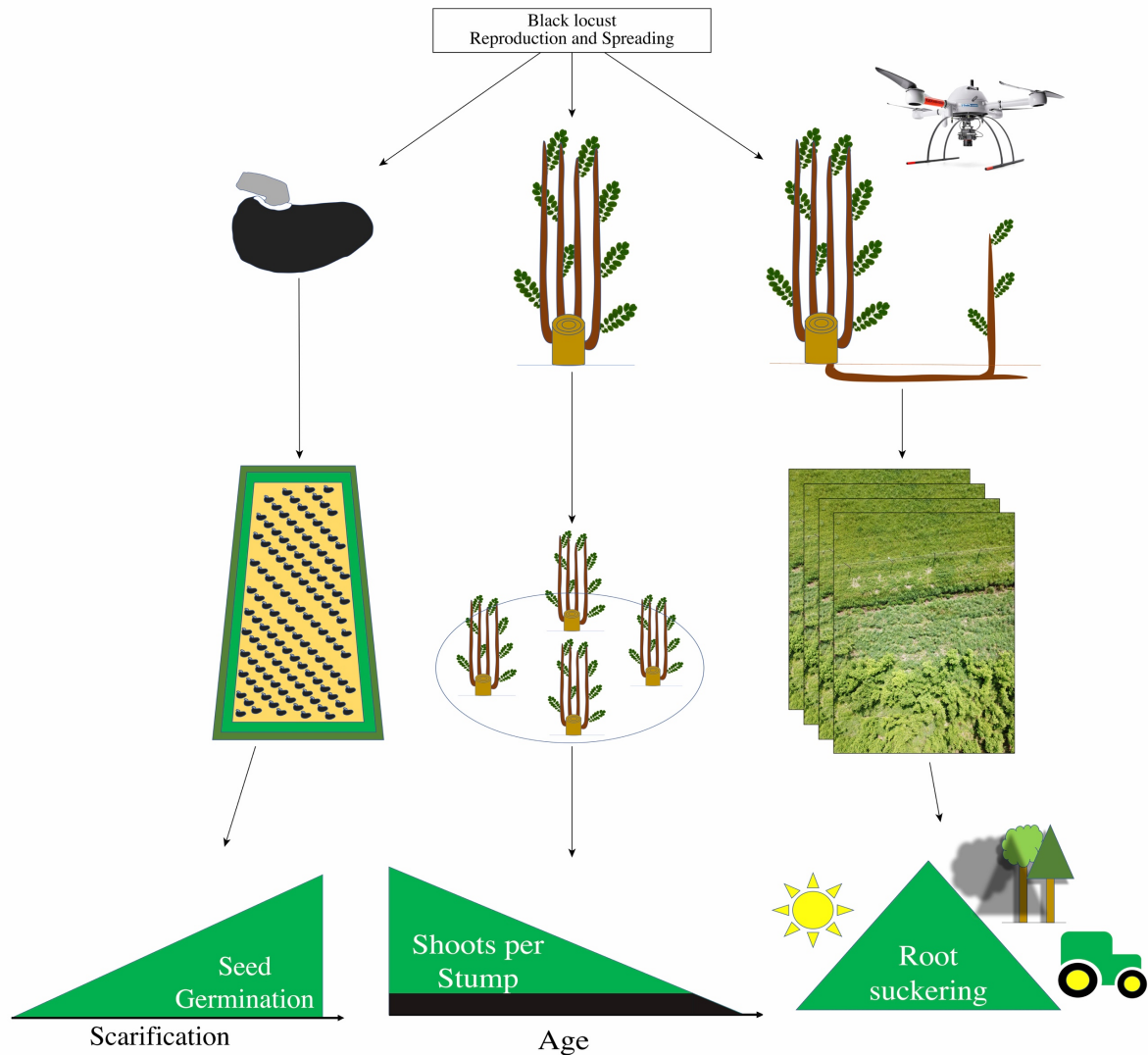


Article 4: Invasiveness Analysis – Risk

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Graphical Abstract



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Article

Robinia pseudoacacia L. in Short Rotation Coppice: Seed and Stump Shoot Reproduction as well as UAS-based Spreading Analysis

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Abstract: Varying reproduction strategies are an important trait that tree species need in order both to survive and to spread. Black locust is able to reproduce via seeds, stump shoots, and root suckers. However, little research has been conducted on the reproduction and spreading of black locust in short rotation coppices. This research study focused on seed germination, stump shoot resprout, and spreading by root suckering of black locust in ten short rotation coppices in Germany. Seed experiments and sample plots were analyzed for the study. Spreading was detected and measured with unmanned aerial system (UAS)-based images and classification technology—object-based image analysis (OBIA). Additionally, the classification of single UAS images was tested by applying a convolutional neural network (CNN), a deep learning model. The analyses showed that seed germination increases with increasing warm-cold variety and scarification. Moreover, it was found that the number of shoots per stump decreases as shoot age increases. Furthermore, spreading increases with greater light availability and decreasing tillage. The OBIA and CNN image analysis technologies achieved 97% and 99.5% accuracy for black locust classification in UAS images. All in all, the three reproduction strategies of black locust in short rotation coppices differ with regards to initialization, intensity, and growth performance, but all play a role in the survival and spreading of black locust.

Keywords: *Robinia pseudoacacia* L.; reproduction; spreading; short rotation coppice; unmanned aerial system (UAS); object-based image analysis (OBIA); convolutional neural network (CNN)

1. Introduction

The spreading of tree species is influenced by overcoming barriers (e.g., geographical) as well as survival and reproduction strategies. Trees are able to reproduce generatively via seeds, and vegetatively as well, for example via stump shoots or root suckers. Nevertheless, little research has been conducted on the reproduction and spreading strategies of black locust in short rotation coppices. However, examining the details within a holistic perspective of reproduction strategies for tree species may improve the estimation of spreading and survival potential, especially of non-native or invasive tree species.

Black locust (*Robinia pseudoacacia* L.) originated in the eastern part of North America, particularly in the Appalachian regions [1,2]. By the early 17th century, black locust had been introduced to Europe [3–6]. Today, black locust appears in many European countries, especially in Hungary, Northern Germany, Western Poland, Czech Republic, Southern Slovakia, and Eastern Austria [6]. In Germany, a change in

energy policies aimed towards a reduction in the use of fossil fuels and greater utilization of renewable energy since 2004 [7] marked an increase in fast-growing tree production, including black locust in short rotation coppices (harvested every 2–20 years) [8,9]. Nevertheless, some studies have declared black locust to be an invasive non-native tree species in Europe [6,10–12]. However, other studies consider black locust to be an alternative tree species, for example for European ash (*Fraxinus excelsior*) [13,14], whose high dieback is caused by the fungal pathogen *Hymenoscyphus fraxineus* [14].

Black locust possesses many growth characteristics that makes it ideal for short-rotation biomass production such as: rapid growth, high drought tolerance, and nitrogen-fixation [15,16], as well as the ability to reproduce via stump shoots in response to harvest. Black locust starts flowering at the age of six years [17]. Many insects benefit from this characteristic, including honeybees [18]. Moreover, the production of black locust honey is very common and economically important, especially in Hungary [17]. A maximum of 15,559 flowers per tree were counted in an eight-year-old black locust plantation [18], and 12,000 seeds/m² were identified in a monodominant stand [6,19]; black locust thus produces an abundance of seeds. As black locust belongs to the *Fabaceae* family, the seed coat is hard and impermeable [20,21]. Hence, seeds require scarification for successful germination [21–23] and priming of seeds is favorable [21,24,25]. Seeds prefer mineral-rich sandy and loamy-sandy soil [17,26,27]. Additionally, seeds are dormant [28] and Voss and Edward [29] observed germination of black locust seeds 88 years after the seeds were collected. Moreover, increasing germination and seedling density was observed in the year immediately following a fire event; it is likely that some seeds survive fire and benefit from the light availability [30–32]. Nevertheless, scarification intensity has not been researched much, particularly in seeds from an eight-year-old short rotation coppice.

Vegetative reproduction strategies of black locust include re-sprouting stump shoots and root suckers. Stump shoot sprouting occurs in many broad-leaved trees in response to harvest or damage and is a characteristic feature of the special forest management practice known as (short rotation) coppicing. Stump shoots typically grow in densely populated stands and often have several shoots per stump. The biomass production differs compared to non-harvested trees, especially for black locust [33]. To account for this, specific yield tables and growth models for coppices of different broad-leaved tree species were developed [34–46]. Moreover, in nutrient-poor and sandy forest stands, a decreasing number of black locust stump shoots with increasing shoot age and a wide variety of stump shoots depending on the stump distance and stump age was observed [47,48]. However, the sprouting intensity and dieback of black locust shoots with increasing shoot age in densely populated short rotation coppices have not been a focus of research up to now.

The spreading of black locust short rotation coppice seems to take place primarily via root suckering [49]. Root suckers are shoots which grow from adventitious buds in roots of trees or shrubs. Crosti et al. [50] observed decreasing spreading intensity of black locust in orchards and nearby forests in Italy. Furthermore, Vítková et al. [6] published an overview of the distribution of black locust in central and eastern Europe on a landscape scale based on inventory data. However, there is a lack of knowledge about the spreading potential of black locust on a small scale, particularly with respect to short rotation coppice. Thereby, tree species detection and spreading could be analyzed via field measurements [50], satellite data [51,52], airplanes, or unmanned aerial systems (UAS) [53–63]. Field measurements are usually time consuming. Satellite data can be used to detect ecosystem structures and changes for large areas [64–68]. However, fine-scale field maps are difficult to generate from satellite or airplane data because they have a low spatial and temporal resolution and are generally expensive. Therefore, UAS allow the detection of ecosystem structures and changes offering a higher spatial resolution of small, specific, and detailed vegetation structures [18,69]. Additionally, UAS have quick turnaround times, are very cost-efficient and are useful supplements to data from satellites, airplanes, terrestrial manual, and other data analyses. Invasiveness analysis using UAS was demonstrated by Lehmann et al. [70] for invasive *Acacia mangium* management in Brazil. Müllerová et al. [71] conclude that the near-infrared (NIR) spectrum is very important for detecting black locust via satellite and UAS. Common approaches to analyzing UAS images are pixel-based

analysis [18] and object-based image analysis (OBIA) [70], primarily including structure from motion (SfM) point clouds [53–55,57,58,60]. Nevertheless, OBIA has not yet been used to analyze the spreading of black locust in short rotation coppices.

Furthermore, there is an increasing interest in machine learning algorithms for data and image analysis, such as the application of the random forest model [52,72–76], support vector machine [73–76], and deep learning algorithms, especially convolutional neural networks (CNNs) [62,73,75,77–79]. However, CNNs were not previously utilized for the classification of black locust in short rotation coppices under varying conditions in single images.

The main purpose of this study is to estimate and monitor the invasiveness potential of black locust. Therefore, in the current study, we analyze the seed germination, stump shoot survival, and spreading of black locust planted in short rotation coppices. We investigate the seed germination of 3000 seeds, focusing on six different seed experiments. After the treatment, the seeds were seeded in planting boxes and watered. Moreover, we estimate black locust stump shoot sprouting and dieback with the aid of sample plots in seven different sample areas, including 5244 stump shoots. For this purpose, the relationship of the planted trees and the current shoots per stump were calculated. Additionally, spreading was estimated in short rotation coppices at a length of 2124 m based on UAS images by using OBIA. Furthermore, a deep learning algorithm was tested to classify black locust under varying light conditions, flying altitudes, UAS, and cameras in single images. This study deals with the following research questions:

- (1) What is the average germination of black locust seeds after six different seed treatments?
- (2) How many stump shoots survive in short rotation coppices depending on shoot age?
- (3) What is the average sprouting distance of black locust, depending on neighboring forest, meadow, farmland, and along a dirt road analyzed via OBIA in UAS images?
- (4) What is the accuracy and loss of black locust classification in single UAS images under varying conditions by using a CNN?

2. Materials and Methods

2.1. Site Description

The ten analyzed study sites were located in northeastern Germany (Table 1, Figure 1). The annual precipitation ranges between 495 mm and 671 mm and the mean annual temperature ranges between 7.4 °C and 9.4 °C [80]. The elevation above sea level is 22 to 149 m. Seeds were collected in winter 2016 in two eight-year-old *R. pseudoacacia* short rotation plantations in Germany (Lauchhammer and Röblingen). Thereafter, the seeds were stored in a seed bank (Thuringia, Germany). Stump shoot sample plots in seven study sites were analyzed between November 2016 and March 2017. The UAS images and field data for the spreading analysis were collected in May 2018 in Grunow-Dammendorf (Germany).

Table 1. Site description including location, longitude (long), latitude (lat), tree/shoot age, and analysis methodology.

Site Abbreviation	Location	Long (°E)	Lat (°N)	Tree/Shoot Age	Analysis
BG	Blumberg	14°10'24"	53°12'25"	3	Stump shoot
BH	Buchholz	12°38'9"	53°15'34"	2	Stump shoot
DA	Grunow-Dammendorf	14°25'5"	52°8'26"	4	Spreading
GU	Gumtow	12°14'11"	52°59'46"	2	Stump shoot
KL	Klein Loitz	14°30'57"	51°36'35"	3	Stump shoot
LH	Lauchhammer	13°50'57"	51°32'20"	8	Seed collection
PA	Paulinenaue	12°43'52"	52°39'44"	3	Stump shoot
RM	Röblingen	11°42'42"	51°25'57"	8	Seed collection
WA	Wainsdorf	13°29'4"	51°24'50"	1	Stump shoot
WZ	Welzow	14°14'7"	51°33'32"	1	Stump shoot

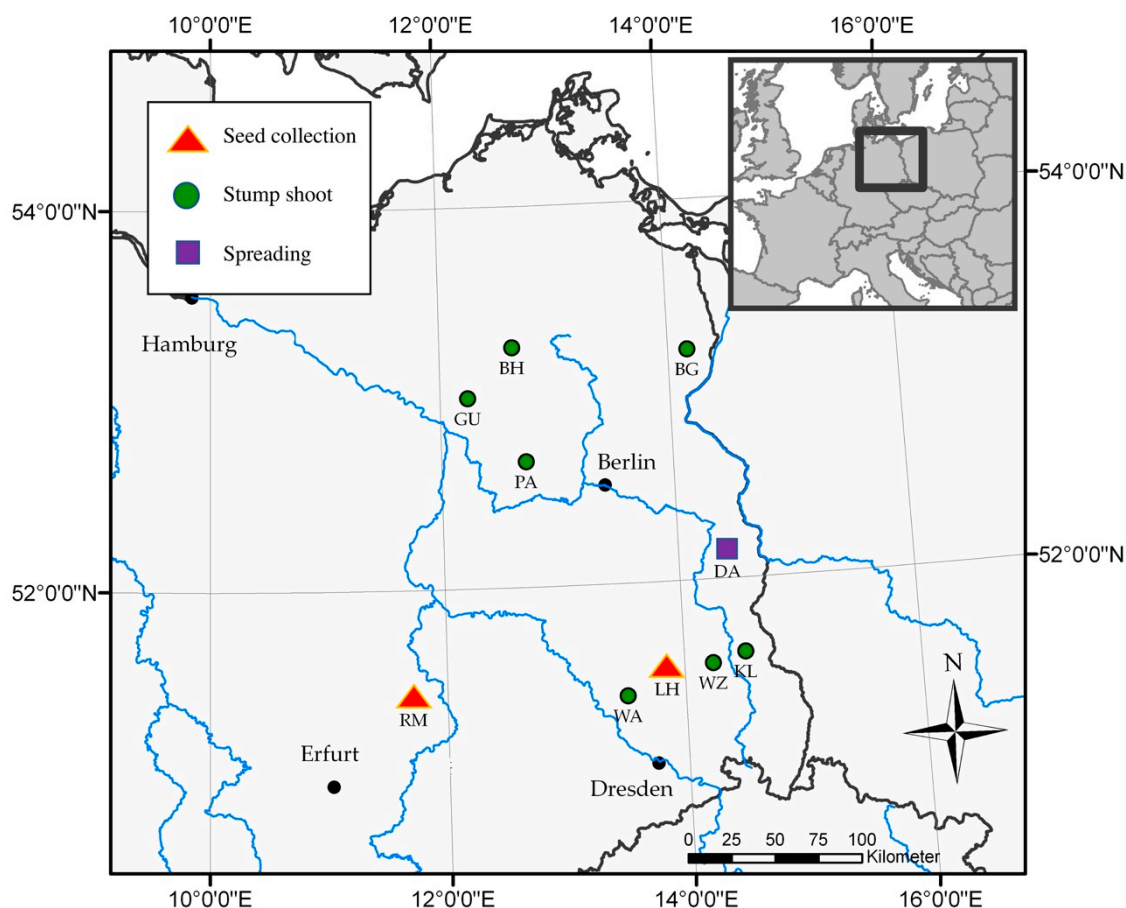


Figure 1. Map of the study area in Europe, indicating the ten *R. pseudoacacia* sampling sites in Germany [81]. Red triangles are sites visited for seed collection, green dots are the sites where sample plots for the stump shoot analysis were measured, and the purple square is the study site of the UAS image analysis, which was applied for the spreading detection. For an overview of site abbreviations, see Table 1.

2.2. Reproduction Analysis

The analysis was divided into three parts (Figure 2) to estimate the reproduction and spreading of black locust: seed, stump shoot, and root suckering analysis. Seed germination was undertaken to determine differences in seed treatments. Sample plots were used for the stump shoot analysis, which was conducted to analyze the number of shoots per stump depending on the shoot age. Spreading was analyzed by using an UAS and image analysis to observe the spreading from a bird's-eye view.

2.3. Seed Germination

The analysis of the seeds started in March 2018 and finished in July 2018. The seeds were seeded in sandy mineral soil in planting boxes. We tested 100 seeds in five iterations for six treatments (3000 seeds):

- (I) seeds were seeded and watered,
- (II) seeds were soaked for 24 h in water at a water temperature of 18 °C (64.4 °F) and then seeded and watered,
- (III) seeds were stored at an air temperature of 45 °C (113 °F) for two hours, and thereafter stored at an air temperature of −20 °C (−4 °F) for a further two hours and then seeded and watered,
- (IV) seeds were stored at an air temperature of 60 °C (140 °F) for two hours and thereafter stored for two hours at an air temperature of −20 °C (−4 °F) and then seeded and watered,

- (V) seeds were scalded with hot water, seeded and watered, and
- (VI) seeds were mechanically scarified, seeded and watered.

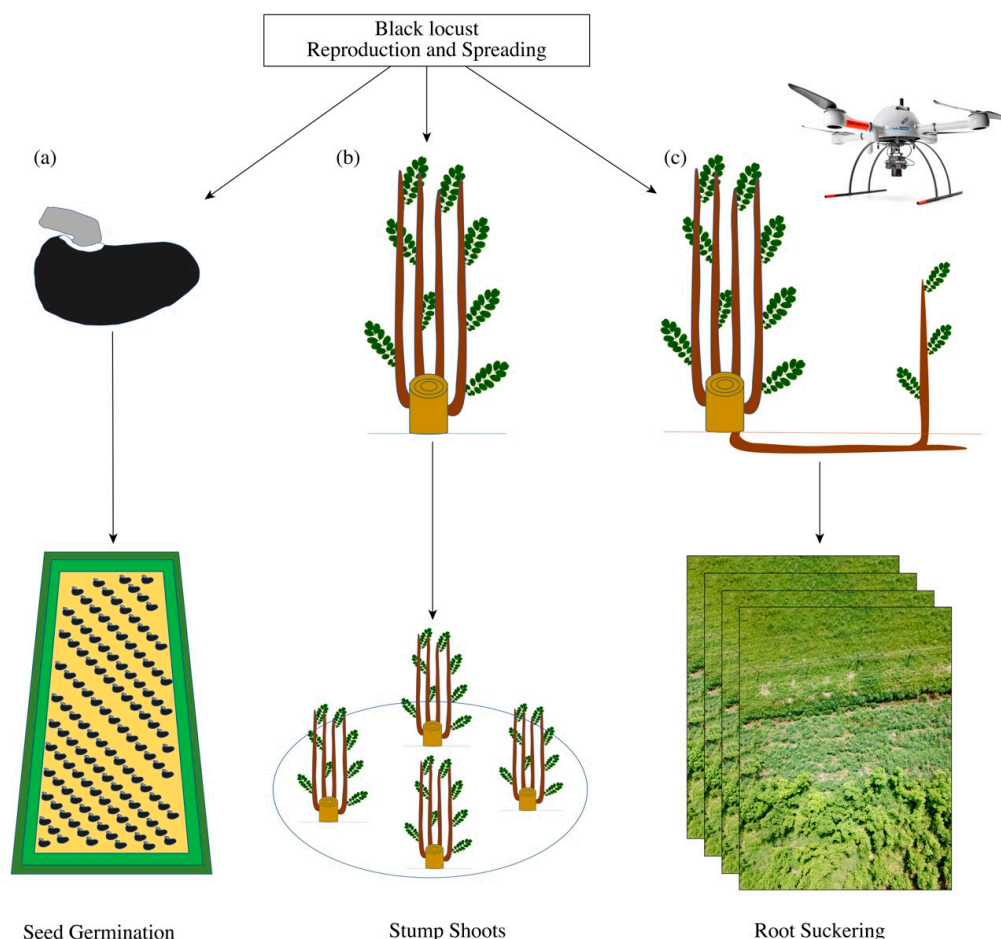


Figure 2. Flow chart showing the reproduction and spreading analysis: seed germination (a), stump shoot analysis (b), and root suckering (spreading) analysis (c). Seeds were seeded in planting boxes, and watered. Stump shoots were analyzed in sample plots. Spreading was estimated with the aid of UAS-images [82].

We calculated the average seed germination, standard deviation (SD) and standard error of the mean (SE) for each seed treatment. Furthermore, we performed an analysis of variance (ANOVA) with the software R [83]. Seed data were (1) variance heterogeneous and (2) normally distributed. Therefore, we applied a one-way analysis of means and pairwise *t*-test for pairwise comparisons [83,84].

2.4. Stump Shoot Analysis

In total, 5244 black locust stump shoots were investigated, measuring leafless stump shoots in 33 sample plots. Sample plots sizes ranged from 4 to 6 m radius and contained a minimum of 150 stump shoots. All stump shoots in the sample plot were counted. Furthermore, the planting distance was listed by the land manager and checked on the study sites. The measured number of stump shoots per sample plot was divided by the number of established/planted black locust trees to calculate the average number of shoots per stump, per site, and per age class. Moreover, we calculated SD, SE, confidence interval (CI) with a default of 95% for each age class and performed an ANOVA [83]. Stump shoot data were (1) variance homogeneous and (2) normally distributed. Hence, we applied an ANOVA as global test and Tukey test for pairwise comparisons [83,84].

2.5. Root Suckering (Spreading)

Two quadcopters were used as a UAS platform: Microdrones MD4-1000 [82] and dji Mavic Pro [85]. The Microdrones MD4-1000 was equipped with two camera systems synchronously for the image collection: a multispectral MAPIR Survey 3 (red, green, NIR) camera [86] with an image size of 4000×3000 and a red-green-blue (RGB) SONY-ILCE-5100 [87] camera with a picture size of 6000×4000 . Furthermore, the dji Mavic Pro took images with the aid of the RGB camera DJI FC220 (picture size of 4000×3000) [85]. Weather conditions were calm, and the lighting conditions varied between sunny and cloudy.

2.5.1. Object-Based Analysis (OBIA)

The analyzed total length was 2124 m, whereby the neighboring areas were comprised of meadow (726 m), farmland (565 m), dirt road (293 m), and pine forest (540 m). For georeferencing purposes, 13 white ground control points were randomly placed. In addition, planting lines were calibrated by using a mobile differential global positioning system [88]. The altitude above ground level was set at 30 m. To create 3D point cloud surface models, the image side and forward overlap were set to 80% [89]. RGB and NIR image data were orthorectified and mosaicked using Pix4D mapper software [90] to create a high-resolution orthoimagery. An SfM approach was used to calculate the digital surface models.

In eCognition Developer software [91], the classification procedure via OBIA consisted of two major steps: (A) segmentation and (B) classification. The multiresolution segmentation was used to aggregate neighboring pixels into segments based on homogeneity criteria (shape, texture, color, compactness, smoothness) and a scale factor (scale parameter) [92]. For the *R. pseudoacacia* classification, the subsequent OBIA was performed using class-specific features. This involved spectral information such as the mean green value, as black locust leaves have a specific light green. Furthermore, the vegetation height (digital surface model–digital terrain model) has the advantage of classifying distinctions of vegetation height for grass as well as trees in plantations and pine forests. To capture black locust trees in the shade neighbor-related pixel values as a contrast to neighboring pixels are important. The resulting classification of *R. pseudoacacia* were exported as shape files into Quantum GIS (QGIS) [93]. Furthermore, during the field survey, marked points of the last planting lines were connected. Starting from this last planting line, five zones from 0 to 10 m were generated with the QGIS software. For each zone, we intercepted and measured the *R. pseudoacacia* cover. To calculate the average distance of spreading (b), the covered area of black locust 0–10 m (A) was divided by the length of the last planting line (a), as shown in Equation (1).

$$b = \frac{A}{a} \quad (1)$$

The accuracy of classification was evaluated by selecting random samples. These randomly selected samples were then manually classified via a visual on-screen interpretation of the available image information together with additional field data. Therefore, 150–200 points were randomly distributed. The object classification was verified among *R. pseudoacacia* and non-*R. pseudoacacia*. Based on the samples, a confusion matrix was produced to evaluate the accuracy of the final classifications, including overall, user's, and producer's classification accuracies and the Kappa Index of Agreement (KIA) [94,95].

2.5.2. Classification via Deep Learning–CNN

A deep learning algorithm on single UAS images was applied to classify black locust and non-black locust. Therefore, 1000 RGB images were selected. Black locust was detected with varying light conditions (sunny and cloudy), flying altitudes (15 m, 30 m, and 100 m), UAS, and cameras (listed in Section 2.5), and thus different structures and colors. In Python [96] via Jupyter Notebook [97] the

tensorflow [98] and keras [99], as well as matplotlib [100], numpy [101], pandas [102], and sklearn [103] libraries were applied for the construction and calculation of the CNN [104,105]. The images were therefore split into train (80%) and test data (20%). The applied CNN structure is presented in Figure 3. In each block, there are convolutional layers and a sub-sampling by max-pooling. In the convolutional layer in the first block, 64 filters with a size of 3×3 were used. In the second block 128 feature channels were integrated, and in the third block 256 feature channels were integrated. The activation function was the rectified linear unit (relu)-function [106]. As an optimization algorithm, we used “Adam” (adaptive moment estimation) [107]. Adam integrates moment and the adaptive learning rate. Furthermore, to avoid overfitting and to allow generalization, we integrated dropouts [106] after each max-pooling and fully connected-layer. The total number of trainable parameters is 4,787,330 per image. We tested our CNN architecture without and with dropout layers: (A) including 6 convolutional layers, 3 max-pooling layers, 1 flatten layer, and 2 fully connected layers; (B) including 6 convolutional layers, 3 max-pooling layers, 1 flatten layer, 2 fully connected layers, and 4 dropout layers (Figure 3). To evaluate the applied CNNs, the loss and the accuracy of the train and test data were computed.

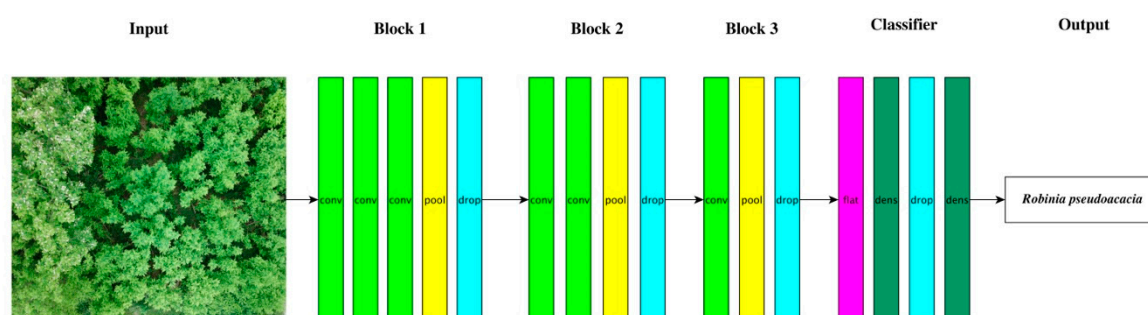


Figure 3. Convolutional neural network (CNN) structure as applied deep learning algorithm. Conv—convolutional layer (light green), pool—max-pooling layer (yellow), drop—dropout layer (blue), flat—flatten layer (pink), dens—fully connected layer (dark green).

3. Results

3.1. Seed Germination

Black locust seed germination after seeding on sandy mineral soils is presented in Figure 4. Seed germination increased with increasing scarification. Hence, seeds directly sown and watered reached 6% germination. By soaking the seeds for 24 h, 8% of the seeds sprouted. By warming the seeds for two hours at 45 °C/60 °C (113 °F/140 °F) and for two hours at −20 °C (−4 °F) as warm-cold variation, the germination reached 23%/69%. Scalding with hot water attained germination of 72% and mechanical scarification resulted in 90% of the seeds sprouting. Moreover, scalding of seeds achieved the highest standard error of the mean (Figure 4). The variance differs significantly (p -value < 0.05) by comparing all applied seed treatments. Nevertheless, the differences were non-significant (p -value > 0.05) between treatments I and II, IV and V, as well as V and VI (Table 2).

Table 2. Pairwise t -tests of the black locust seed germination by (I) directly sowing, (II) 24 h soaking, (III) warm-cold treatment for two hours at 45 °C and two hours at −20 °C, (IV) warm-cold treatment of two hours at 60 °C and two hours at −20 °C, (V) hot water scalding, and (VI) mechanical scarification.

Treatment	I	II	III	IV	V
II	0.3365				
III	<0.001	<0.001			
IV	<0.001	<0.001	<0.001		
V	0.0086	0.009	0.015	0.750	
VI	<0.001	<0.001	<0.001	0.011	0.274

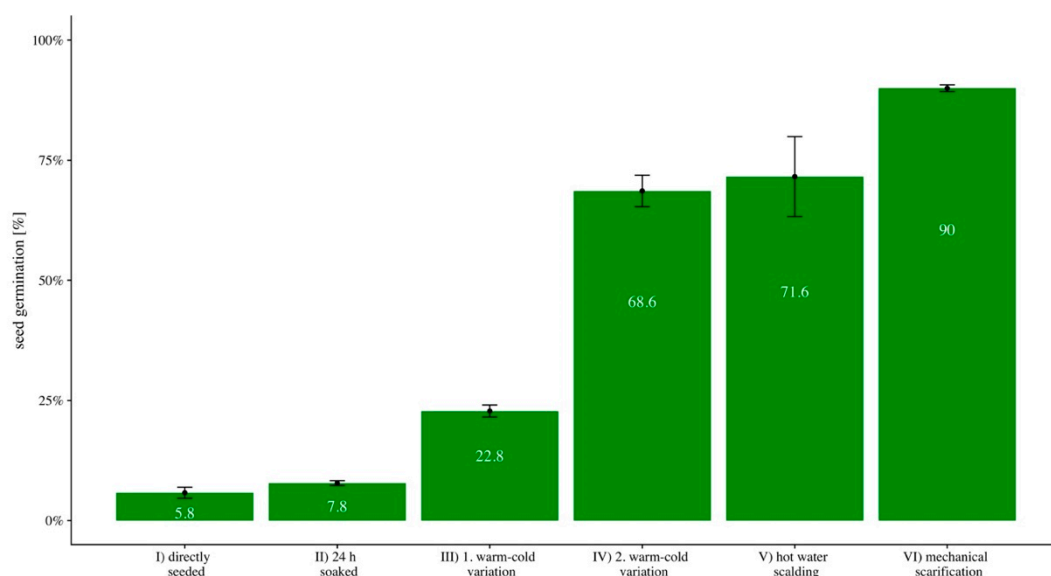


Figure 4. Black locust seed germination (%) by (I) directly sowing, (II) 24 h soaking, (III) warm-cold treatment for two hours at 45 °C and two hours at −20 °C, (IV) warm-cold treatment of two hours at 60 °C and two hours at −20 °C, (V) hot water scalding, and (VI) mechanical scarification. The error bars show the standard error of the mean per seed treatment. Database = 3000 seeds.

3.2. Stump Shoot

The average number of shoots per stump decreases with increasing age (Table 3 and Figure 5). Thereby, the average number of shoots per stump is 4.17 one year after the last harvest, shoots aged 1 year. Two years after the last harvest, an average of 3.61 shoots per stump are alive. In the third year after harvest, the value decreased to 2.18 shoots per stump. The values of the SD, SE, and CI are largest in the 3-year age class and smallest in the 2-year age class. Furthermore, there was a statistically significant difference between age classes as determined by ANOVA (Table 3). Nevertheless, the differences were non-significant between age classes 1 and 2 (p -value 0.313), but significant between age classes 1 and 3 (p -value <0.001) and between age classes 2 and 3 (p -value 0.008).

Table 3. Average number of shoots per stump and per age class as well as the plot and shoot database.

Site Abbreviation	Shoot Age	Plots	Data	Average Shoots	Shoots Per Age	SD	SE	CI	<i>p</i> -Value
WA	1	4	680	2.87	4.17	1.12	0.32	0.71	<0.001
WZ		8	1331	4.82					
BH	2	6	1000	3.98	3.61	0.61	0.16	0.35	
GU		8	1314	3.34					
BG	3	3	441	1.56	2.18	1.19	0.45	1.10	
KL		3	478	3.38					
PA		1	167	0.44					

SD: standard deviation; SE: standard error of the mean; CI: confidence interval (default 95%).

3.3. Root Suckering (Spreading) via OBIA

In Table 4 and Figure 6, the proportion of black locust classified by OBIA in the five zones is shown in percentage depending on the surrounding area: meadow, farmland, dirt road, and pine forest. Furthermore, the average distance is presented in the last row. The further away from the last planting line, the lower the proportion of black locust. Average spreading is highest to dirt road and meadow.

Table 4. Zones 0–2 m, 2–4 m, 4–6 m, 6–8 m, 8–10 m and the average distance as spreading of black locust depending on the surrounding area: meadow, farmland, dirt road, and forest.

Zone	0–2 m (%)	2–4 m (%)	4–6 m (%)	6–8 m (%)	8–10 m (%)	Average Distance (m)
Meadow	43.99	32.37	14.86	3.00	0.33	1.89
Farmland	27.45	19.60	9.78	0.91	0.07	1.16
Dirt road	41.96	37.81	26.07	6.38	0.58	2.26
Forest	40.23	25.42	7.79	1.14	0.002	1.49

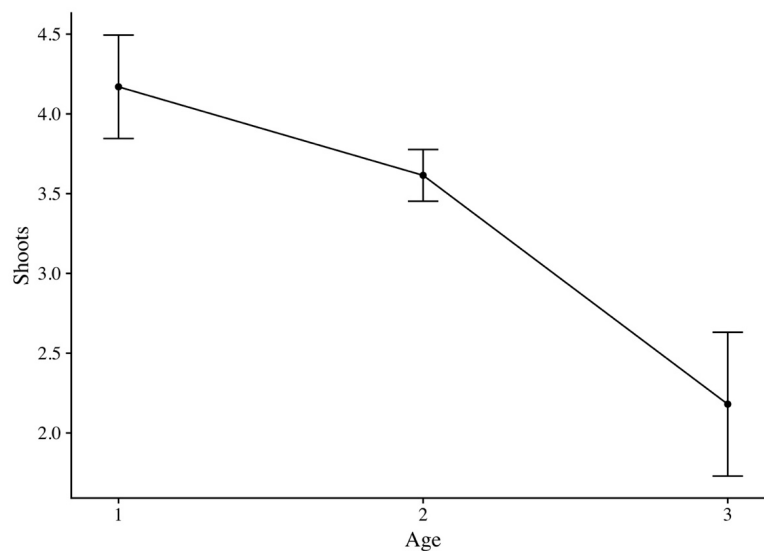


Figure 5. Average number of shoots per stump in relation to the shoot age and the standard error of the mean (data = 5244 shoots). Detailed values are presented in Table 3.

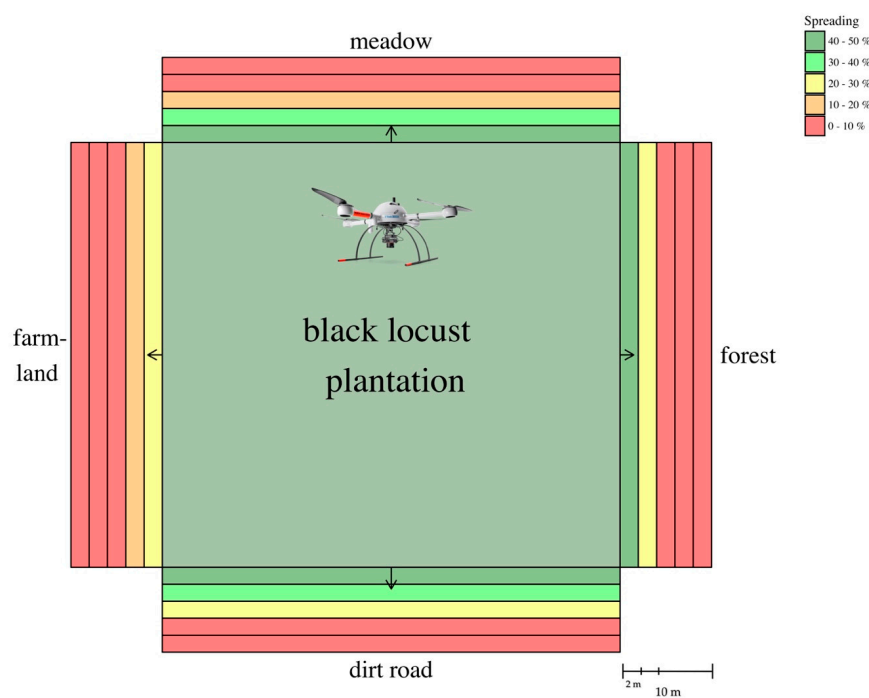


Figure 6. Spreading: outline average values of the areas; 0–2 m, 2–4 m, 4–6 m, 6–8 m, 8–10 m depending on the surrounding areas: meadow, farmland, dirt road, and pine forest. The spreading depends on the proportion of black locust; dark green stands for 40%–50%, light green indicates 30%–40%, yellow is 20%–30%, orange 10%–20% and red 0%–10%. The arrows show the average spreading distance of black locust. For an overview of the results, see Table 4. Database = 2124 m [82].

At 43.99%, the highest value in the 0–2 m zone is reached if the surrounding area is meadow, and the smallest, 27.45%, if the surrounding area is farmland. In the following four zones 2–10 m, the highest black locust quantity is measured if the surrounding area is a dirt road. Generally, the spreading in the first 2 m from the last planting line to the neighboring area is between 40%–50% if the neighboring area is meadow, dirt road, and forest. In the zone from 4–10 m, the proportion is around 0%–10% if the neighboring area is forest or farmland, and in the zone from 6–10 m, the proportion is 0%–10% if the neighboring area is meadow or dirt road. Furthermore, the highest average distance, 2.26 m, is reached if the surrounding area is a dirt road (arrow in Figure 6). At 1.89 m, the second highest average distance is measured if the surrounding area is meadow. In the case of the forest as the surrounding area, the average distance is 1.49 m. The smallest average distance, 1.16 m, is reached if the surrounding area is farmland. The overall accuracy of the OBIA analysis is 0.97 and the overall KIA is 0.93 (Table 5). The producer and user accuracy ranged between 0.96 and 0.98.

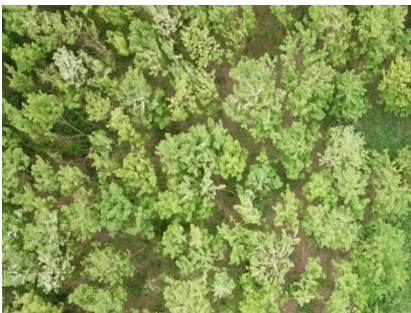
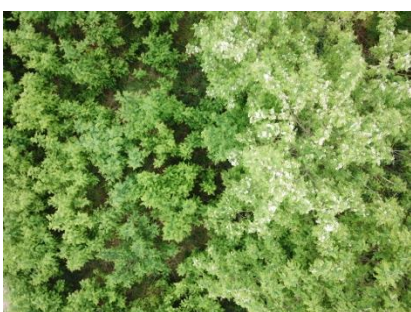
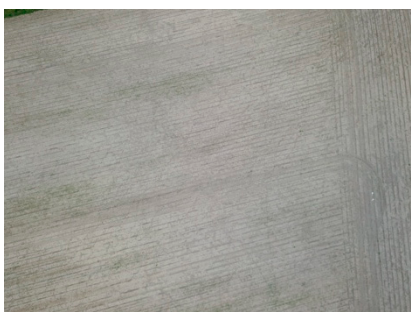
Table 5. Confusion matrix of the classification *R. pseudoacacia* and non-*R. pseudoacacia* via object-based image analysis (OBIA). KIA stands for Kappa Index of Agreement.

		Actual	
		<i>R. pseudoacacia</i>	Non- <i>R. pseudoacacia</i>
Predicted	<i>R. pseudoacacia</i>	177	7
	Non- <i>R. pseudoacacia</i>	5	174
Producer Accuracy		0.975	0.962
User Accuracy		0.963	0.972
KIA Class		0.942	0.928
Overall Accuracy		0.966	
KIA Overall		0.932	

3.4. Classification via CNN

The CNN architecture without dropout layers (A) reached a 90% test accuracy and a 99.7% train accuracy. The test loss was 0.395 and the train loss achieved 0.009. The model of the CNN (B) used for classifying *R. pseudoacacia* and Non-*R. pseudoacacia* is presented in Table 6. On the left-hand side are image examples of test data of *R. pseudoacacia* (1) classifications with varying flying altitudes, light conditions, UAS, and cameras. On the right-hand side are examples of test data of Non-*R. pseudoacacia* (0) category. The CNN's (B) accuracy (Figure 7) in classifying *R. pseudoacacia* (1) and Non-*R. pseudoacacia* (0) increased quickly. After a small number of iterations, the accuracy of the training and validation dataset increased by over 90%, ranging between 90 and 100% from iteration 5 to 50. The training accuracy reached 99.7% and the test accuracy 99.5%. The training and validation loss reduced from 0.7 to a range between 0 and 0.2. Finally, the training loss was 0.009 and the test loss 0.027. The time for fitting the model was 24.2 min, with 3.83 billion training parameters included.

Table 6. Classification examples of the test data set; *R. pseudoacacia* ((a), category 1) and Non-*R. pseudoacacia* ((b) category 0): meadow, dirt road, pine forest, and farmland. Pred is the abbreviation for Prediction. True and the green color of the labels show that the prediction (output in Figure 3) and the class (input in Figure 3) are equal.

(a) <i>Robinia pseudoacacia</i> L.		(b) Non <i>Robinia pseudoacacia</i> L.	
	1 True 1 Pred		0 True 0 Pred
	1 True 1 Pred		0 True 0 Pred
	1 True 1 Pred		0 True 0 Pred
	1 True 1 Pred		0 True 0 Pred

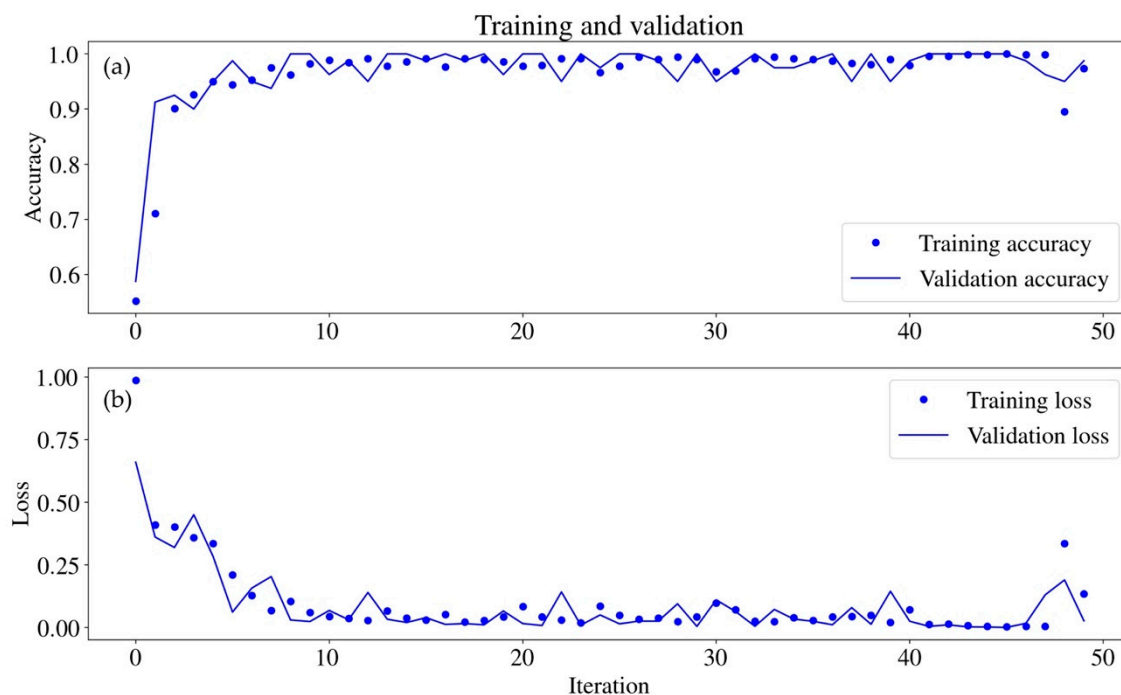


Figure 7. Accuracy (a) and loss (b) of the training and validation (=testing) data for classification of *R. pseudoacacia* and Non-*R. pseudoacacia*, depending on the iterations. Dataset = 1000 images; 80% for training and 20% for testing.

4. Discussion

Reproduction strategies of tree species are an important basis for the survival in spatially and temporally varied ecosystems. However, the reproduction and spreading of black locust in short rotation coppices in Germany have not been analyzed until now. In this study, by applying seed experiments, stump shoot analysis, and spreading measurements, we create an overview of the invasiveness potential of black locust in short rotation coppices in a temperate climate zone. Black locust reproduces generatively via seeds and vegetatively via stump shoots and root suckers. These three reproduction strategies improve the survival of black locust in many ecosystems globally [6,17,108–114]. Thereby, the reproduction and spreading increases with greater damage to the seed coat and the harvest of black locust trees. Further favorable conditions are sandy soil and high light availability.

Reproduction via seeds is a successful strategy for plants globally. Black locust seeds have a hard and impermeable seed coat [20,21]. In the present study, we observed that seed germination increased with increasing mechanical scarification. This is in line with Vines [22] and Redei et al. [23]. Moreover, the seed experiment shows that germination increases when the warm-cold variation is increased. It might be possible that when the warm-cold variation increases, micro-cracks appear, water enters, and light stimuli facilitates the growth processes. During mechanical scarification, the seed's coat is directly damaged and the seedling's development can start. Tompa and Szent-Istvan [115] conclude that hot water treatment was less effective for black locust seed germination. In our study, seeds soaked for 24 h had a 2% higher germination compared to the directly seeded category, with a 6% germination. However, there was no statistically significant difference between seed treatments I) directly seeded and II) soaked in water (18 °C/64.4 °F) for 24 h. However, scalding with hot water yielded the second highest average germination value, with germination above 70%, as well as the highest standard error of the mean. The reason could be the appearance of microcracks in some seed coats and for others the scalding stimulus might be too weak, as the seed coat permeability varied. The applicability of scalding for increasing germination is in line with Velkov [25], as well as with Bogoroditskii and Sholokhov [24]. All in all, it is important to crack and open the seed coat for successful germination.

We have to stress that seeds were collected in winter 2016 and only from two former mining areas, as the black locust trees were non-harvested, aged 8 years, and abundant seeds were collectable. It might be possible that the seed quality and germination from different former types of utilization, sites, stand ages, and years vary, as it is described by Redei et al. [17]. Therefore, depending on the seed samples, the absolute results may differ. However, the relative results might be similar. It is important to note, that flowering and reproduction via seed usually starts at the age of six years [17]. For younger short rotation coppices, the reproduction via seeds might be neglectable. Further research could focus on additional seeds selected at different sites, such as urban sites, pure and mixed forests, and on varying age, years, etc. The seed experiments could be planned as field experiments to analyze the influence of fire [31], landscape destruction [31,49], dryness, insects, diseases [116], storms, landslides [32], etc.

Building stump shoots after being damaged or harvested is a survival strategy of many broad-leafing tree species. As black locust is able to build stump shoots, grows fast, and can grow on nutrient-poor sites, this species was planted in short rotation coppices in Germany [33]. In this study, we estimate that the number of shoots per stump decreases with increasing shoot age, which differs significantly by comparing shoot age classes 1 and 3 as well as 2 and 3. This age–shoot relationship has already been observed for black locust stump shoots in forest stands [47,48]. Zeckel [47] counted 13 shoots on average, and Ertle et al. [48] observed seven shoots per stump one year after harvest, six stump shoots in the second year after harvest [47,48], and four shoots per stump in the third and fourth years after harvest [48]. Those values are higher compared with the stump shoot analysis of the present study. In short rotation coppices, we observed on average 4.2 shoots per stump one year after harvest, 3.6 shoots per stump two years after harvest, and 2.2 shoots per stump three years after harvest. One reason for the difference in the number of shoots per stump in forests compared with short rotation coppices might be the planting distance and the number of trees per hectare. Forests were planted with 2000–3000 trees per hectare, and short rotation coppices typically with 8000–10,000 trees per hectare [9]. This fact corresponds with further observations that black locust mortality increases in plantation and reclamation projects with higher density planting [117,118]. Furthermore, the range in number of shoots per stump widens as stump age and dieback of stumps increases [47,48]. Additionally, significant differences were recorded among black locust biomass production of trees (non-harvested) and stump shoots (harvested) [33] in short rotation coppices. This study is a further step in understanding the relationships of the average shoot sprout and dieback with increasing shoot age. The intensity of dieback and growth partitioning might also depend on the aboveground and belowground resources. As belowground resources, water and phosphorus were described as key drivers for competition among black locust trees and stump shoots [119]. Accordingly, by increasing water and phosphorus availability, the competition among stump shoots and the dieback of shoots increases. Additionally, the fact that the increasing shoot age correlates with a decrease in the number of shoots per stump is important for managers of short rotation coppices, as the wood biomass (quality) increases, but the shoots per stump (quantity) decreases. It could be interpreted that it might be effective to plant black locust at greater planting distances, as more shoots will survive in the first years after harvest. However, this feature could be also interpreted as a risk-minimizing and quality-improving aspect. The slenderness is reduced and the risk of breaking due to wind or weight (for example, abundant seeds) is decreased. The wood quality of the surviving shoots increases as the relative bark proportion decreases and the relative wood proportion per shoot increases. Furthermore, black locust wood differs among juvenile and adult wood [120–123]. Hence, as the black locust tree or shoot becomes older, the wood quality could improve for energy use and construction timber. Further studies might focus on the best planting distances for black locust to improve the target biomass and wood production.

As a third reproduction strategy, black locust is able to spread via root suckering. We observed that the spreading increases with increasing light availability and reduced tillage. The proportion and average spreading distance are highest if the surrounding area is a dirt road or meadow. The proportion and distance were lower if the neighboring area is a forest or farmland. The lower spreading distance

to the farmland could be due to tillage by the land owner and the resulting destruction of the spreading black locust roots. The reason for lesser spreading in the neighboring area if it is a forest could be the light limitation due to the higher pine trees. As a light-demanding tree species, black locust growth is reduced if the light availability is low [124]. This is in line with previous findings discussed in Crosti et al. [50], who found that the density of black locust regeneration in Mediterranean ecosystems is strongly affected by the land cover. Accordingly, abandoned agricultural land is most prone to black locust colonization. The spread was lowest in a zone with orchards. This zone was described as very effective for controlling black locust invasion [50]. Moreover, our study shows that independent of the neighboring area, the proportion of black locust cover and spreading decreases, the longer the distance is to the plantation. Therefore, it seems that the spreading is a step-by step-process. Nevertheless, it has been found that black locust is able to spread locally up to 1 m per year [125]. Huntley [2] and Grese [126] describe that root sprouting usually begins when plants are 4 years old and increases rapidly in full sun, open areas, and particularly in sandy soil [126]. As with stump shoots, sprouting is a response to disturbance, and sprouts need sufficient light to survive [32]. Moreover, as black locust belongs to the family *Fabaceae*, it is able to fix atmospheric nitrogen via rhizobia [15,16], which results in an increasing nitrogen concentration and a change in the chemical compositions in the soil [127–129]. In Europe, particularly in Germany, nature conservation areas are often open areas, such as dry and semi-dry grasslands, which belongs to the most species-rich and endangered types of habitats [130]. To protect such areas and avoid a black locust spreading, it is important to avoid planting black locust close or next to protected areas. Long-term investigations might provide answers regarding further ecosystem modifications of black locust. Moreover, on a global scale, the spreading of atmospheric nitrogen fixing tree species is increasing [6,70,131]. This might be due to anthropogenic influences such as an increased CO₂ and nitrogen concentration in the atmosphere [132,133]. Additionally, the nitrogen concentration often increases locally due to the usage of fertilizer on farmlands [134]. The increasing spreading of atmospheric nitrogen fixing tree and shrub species that results in ecosystem-changing processes by enriching the soil with nitrogen, seems to be a manmade issue. Moreover, increasing temperature, reduced frost [2,135,136], and the dieback of tree species, such as European ash [13,14], could result in a further increase in black locust and additional non-native tree species. It is important to stress that the analyzed length to the neighboring areas varies and that mother stumps are missing in a few planting lines, which might influence the results. Therefore, to validate the results further, black locust stands with other neighboring ecosystems could be investigated.

The approach of combining UAS images with OBIA, as described in the present study, could be the foundation to create a database to appraise the spreading and ecosystem changes caused by *R. pseudoacacia* and could be further applied to non-native and native tree species. To this end, UAS allows monitoring with a higher spatial resolution of small, specific, and detailed vegetation structures [18,69,137] and avoids environmental damage. Another advantage of UAS images is the possibility to generate 3D surface models [69]. The 3D surface models in this study were important for the classification of the vegetation height of *R. pseudoacacia*, grass, and forest pine trees. The height of the black locust trees in the analyzed plantation ranged between 0.2 and 3.0 m. This height classification approach is common for tree analysis [53–55,57,58,60]. However, a limitation of current UAS images is that depending on stand density only the top of the tree or stump shoot is detectable [138]. Solutions could be the integration of high-resolution light detection and ranging (LiDAR) systems to an UAS [139] or UAS flying below the canopy as a possibility for future technology. Both could detect the deeper canopy and stand layers [139]. Along with the vegetation height, the neighbor-related pixel values and contrast were important for detecting black locust trees in shade caused by higher vegetation or cloud cover. Furthermore, the mean green values were considerable, as *R. pseudoacacia* leaves have a specific light green. The advantage of OBIA compared to pixel-based analysis is the avoidance of misclassification of single pixels [140,141]. OBIA includes geometric properties such as dimension and texture of the target tree species and creates additional options as compared to pixel-based analysis [92,142]. Nevertheless, the OBIA approach as applied in the present study relies on a labor-

intensive survey, which needs expertise in image converting. This is generally time consuming and expensive [143,144]. Furthermore, mistakes due to human operation are possible [145].

In addition, machine learning algorithms are increasingly being used for data and image analysis [52,62,72–79]. The CNN applied in the current study was tested to classify single black locust images under varying conditions and attained a high test accuracy of 99.5%. However, disadvantages of CNN algorithms are that a high number of labeled training images should be available [146], as well as the difficult traceability of the used classification features [105]. Nevertheless, studies [73,145,146] have shown that when the training sample size was high, CNN tended to show better results and accuracies compared to random forest, support vector machine, and fully convolutional networks. Therefore, Liu and Abd-Elrahman [146] used 400 UAS images per object, Diegues et al. [147] applied about 700 underwater images, Abrams et al. [148] operated with 700 canopy and 800 understory images per habitat class as well as Li et al. [73] used 5,000 satellite images per category. In the present study 1000 UAS images were selected to classify black locust and non-black locust. Moreover, varying CNN architectures are existing and being further developed. We evaluate the classification accuracy of two widely used CNN architectures: (A) including 6 convolutional layers, 3 max-pooling layers, 1 flatten layer, and 2 fully connected layers as simplified VGG architecture [104]; (B) including 6 convolutional layers, 3 max-pooling layers, 1 flatten layer, 2 fully connected layers, and 4 dropout layers, which is similar to AlexNet architecture [106]. Our proposed approach (B), which includes dropout layers, achieved a higher test accuracy at 99.5% compared with the applied VGG architecture without dropout layers, which achieved a 90% test accuracy. Both CNN architectures achieved a 99.7% training accuracy. Therefore, the applied VGG architecture tend to overfit the model. Nevertheless, the applied dropout layers [106] in the applied AlexNet architecture avoided overfitting and allowed generalization with a 99.5% accuracy by modelling 1,000 images. This is in line with previous findings discussed in Li et al. [73], who found that the AlexNet architecture achieved the highest accuracy in oil palm tree detection (satellite images) compared to LeNet [149] and VGG-19 architecture. Single image classification of black locust, as applied in the present study, could be advanced in further studies by applying CNN architectures for segmentation (e.g., semantic or pedestrian segmentation) [62,145,150–152]. It is important to stress that the described CNN architectures were tested on images of black locust in a short-rotation coppice at one site in a variation of flying altitudes (15 m, 30 m, and 100 m), light conditions (sunny and cloudy), UAS (Microdrones MD4-1000 [82] and dji Mavic Pro [85]), and cameras (SONY-ILCE-5100 [87] and DJI FC220 [85]). For a comprehensive training and the applicability to different sites, further images of black locust from different age classes, health conditions, seasonal change of leaf colors (e.g., spring and autumn in Central Europe), tree heights, stand densities, competition [119], multiple mixed stands, environments, drones, etc., are needed, but this methodology offers a new possibility for faster and automatic detection of black locust and other tree species.

This study focuses on the reproduction of black locust in short rotation coppices in Germany in order to improve the estimation of the spreading and survival potential. The analysis of generative and vegetative reproduction shows that scarification increases germination, the numbers of shoots per stump decrease with time, and sprouting is influenced by light availability. Our research study gives an overview of the invasiveness potential and reproduction strategies of black locust in short rotation coppices, which is important for managing black locust effectively, for example for biomass production and nature conservation.

5. Conclusions

This study provides an overview of the reproduction strategies of black locust by analyzing the reproduction and spreading in ten short rotation coppices in Germany. The seed experiments focus on six different treatments in five iterations and show that seed germination increases with increasing warm-cold variation (23%–69%), hot water scalding (72%), and mechanical scarification (90%) of the hard and impermeable seed coat. Furthermore, after scalding with hot water, the seed

germination reached the highest standard error of the mean. Moreover, the findings showed that the seed germination is less than 10% when seeds were directly seeded or soaked in water (18 °C/64.4 °F) for 24 h. Stump shoots were counted in sample plots in varying age classes (1–3 years). The numbers of shoots per stump decrease as shoot age increases, which differs significantly by comparing age classes 1 and 3, as well as 2 and 3. Spreading of root suckers was analyzed with the aid of UAS platforms and image analysis via OBIA. The spreading distance increases with increasing light availability and is decreases with tillage. Thereby, the proportion and average spreading distance are highest if the surrounding area is a dirt road or meadow. The proportion and distance were lower if the neighboring area is a forest or farmland. Furthermore, we tested a CNN model to classify black locust under varying conditions in single images (1000 images) and achieved a high accuracy of 99.5% by including 6 convolutional layers, 3 max-pooling layers, 1 flatten layer, 2 fully connected layers, and 4 dropout layers. The methodology and results presented herein provide local managers, foresters, and scientists with the opportunity to estimate reproduction and spreading of black locust and other tree species.

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Abbreviations

The following abbreviations are used in this manuscript:

Adam	Adaptive moment estimation
ANOVA	Analysis of Variance
CI	Confidence Interval
CNN	Convolutional Neural Network(s)
h	Hours
KIA	Kappa Index of Agreement
LiDAR	Light Detection and Ranging
NIR	Near-InfraRed
OBIA	Object-Based Image Analysis
QGIS	Quantum GIS
RGB	Red-Green-Blue
SD	Standard Deviation
SE	Standard Error of the Mean
SfM	Structure from Motion
UAS	Unmanned Aerial System(s)

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