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The influence of soil treatments on post fire regeneration of native vegetation in a context of reforestation in an arid landscape in southern Portugal

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Thesis submitted in partial fulfillment of the requirements for the master's degree of Environmental Sciences and Management - Environmental Monitoring

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SUMMARY

ACRONYMS

ANOVA	Analyse of variance
BS	Between swales
C. ladanifer	Cistus ladanifer
Cistus H	Cistus height
Cistus NGH	Cistus new growth height
Cistus OGH	Cistus old growth height
Cistus M	Cistus humidity
Cistus DW	Cistus dry weight
Cistus SD	Cistus stem diameter
Cistus PA	Cistus abundance
Cistus NbB	Cistus number of branches
Cistus LA	Cistus leaf surface area
Cistus LSA	Cistus specific leaf surface area
Cistus WW	Cistus wet weight
csv	Comma separated values
DB	Dry biomass
RGB	Red-green-blue
GVC	Green vegetation cover
OS	On swales
PC	Principal component
PCA	Principal component analysis
PM	Plant moisture
Soil AC	Soil after cooling
Soil BC	Soil before cooling
Soil FW	Soil fresh weight
Soil DW	Soil dry weight
SM	Soil moisture
SOM	Soil organic matter
SR	Survival rate
ST	Stones with a diameter > 2mm
WW	Wet weight

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ABSTRACT

Wildfires have increased in intensity and extent in Portugal over the past decade. Such events significantly alter ecosystems and negatively affect soil properties and vegetation cover, leading to erosion, nutrient loss, and vegetation depletion. Under such poor conditions, human interventions to improve soil richness are often needed to accelerate forest recovery and native vegetation regeneration. The objective of this study is to evaluate the effects of soil treatments on native vegetation and their impact on the survival of seedlings planted for reforestation purposes. As part of a broader research project, several soil treatments were applied on recently burned soil: control zone; prior vegetation removal; creation of contour bunds; creation of contour bunds and woodchips disposal; creation of contour bunds and green waste compost disposal. This research will focus on addressing (1) the influence of each treatment on soil properties of which soil organic matter (SOM) and soil moisture (SM); (2) the influence of soil treatments on native vegetation biomass, cover, and moisture and their interactions with soil properties; (3) the impact of the presence and development of native vegetation on the survival rate of tree and shrub seedlings planted on site. Particular attention will be pay to the effects of the treatments on one native species, *Cistus ladanifer* by examining its abundance and some of its phenological parameters. Finally, (2bis) this work investigated the development of a simple tool to assess biomass in the field using green vegetation cover as a proxy.

The results showed that soil organic matter is higher where there have been no treatments or only vegetation removal. This is likely due to (i) field preparation, which may have removed burned organic materials and turned over the soil layer, and (ii) higher vegetation cover in these plots. Biomass and cover of native vegetation are also higher in these plots, likely due to (i) vegetation removal by machinery and (ii) the layer of supplemental organic material (woodchips or compost) preventing plant growth. Native vegetation is more humid where contour bunds were created, likely due in part to (i) increased infiltration rates and rooting depth, and (ii) less abundant vegetation and lignified species. *Cistus ladanifer* grows more humid, taller, and with larger leaf areas in the same plots, indicating that this species may be a good indicator of treatment effects. There is a negative correlation between the presence of native vegetation (including *Cistus ladanifer*) and the survival rate of trees and shrubs, indicating that native vegetation may compete with them. However, it is more likely that the planted trees and shrubs survived better thanks to the positive effect of the organic amendments, which simultaneously reduced vegetation cover. Finally, a linear regression between biomass and GVC showed that GVC is a good proxy for biomass estimation.

RÉSUMÉ

Les incendies de forêt ont augmenté en intensité et en étendue au Portugal au cours des dernières décennies. Ces événements climatiques extrêmes modifient considérablement les écosystèmes et ont un impact négatif sur les propriétés du sol et la couverture végétale, une érosion et une perte de nutriments dans les sols ainsi que l'appauvrissement de la végétation. Dans de telles conditions, une intervention humaine est souvent nécessaire pour améliorer la richesse du sol et accélérer la reconstitution des forêts et la régénération de la végétation native. L'objectif de cette étude est d'évaluer les effets des traitements du sol sur la végétation indigène et leurs impacts sur la survie des semis plantés à des fins de reforestation. Dans le cadre d'un projet de recherche plus large, plusieurs traitements du sol ont été appliqués sur un sol récemment brûlé : zone témoin ; enlèvement préalable de la végétation ; création de digues de contour ; création de digues de contour et application de copeaux de bois ; création de digues de contour et application de compost vert. Cette recherche se concentrera sur (1) l'influence de chaque traitement sur les propriétés du sol, dont la matière organique du sol (SOM) et l'humidité du sol (SM) ; (2) l'influence des traitements du sol sur la biomasse, la couverture et l'humidité de la végétation native et leurs interactions avec les propriétés du sol ; (3) l'impact de la présence et du développement de la végétation indigène sur le taux de survie des semis d'arbres et d'arbustes plantés sur le site. Une attention particulière sera accordée aux effets des traitements sur une espèce indigène, *Cistus ladanifer*, en examinant son abondance et certains de ses paramètres phénologiques. Enfin (2bis) ce travail a été le développement d'un outil simple pour évaluer la biomasse sur le terrain en utilisant la couverture végétale verte comme proxy.

Les résultats ont montré que la matière organique du sol est plus élevée là où il n'y a eu aucun traitement ou seulement une suppression de la végétation. Cela est probablement dû (i) à la préparation du terrain, qui peut avoir enlevé les matières organiques brûlées et retourné les couches de sol riches en carbone, et (ii) à une couverture végétale plus élevée dans ces parcelles. La biomasse et la couverture de la végétation native sont également plus élevées dans ces parcelles, probablement en raison (i) de l'enlèvement de la végétation par les machines et (ii) de la couche de matière organique (copeaux de bois ou compost) qui empêche la croissance des plantes. La végétation native est plus humide là où des digues de contour ont été créées, probablement en partie à cause (i) de l'augmentation des taux d'infiltration et de la profondeur d'enracinement, et (ii) d'une végétation moins abondante et d'une abondance plus importante en espèces lignifiées. Les *C. ladanifer* récoltés sont plus humides, plus grands et présentent une surface foliaire plus importante dans les mêmes parcelles, ce qui indique que cette espèce peut être un bon indicateur des effets du traitement. Il existe une corrélation négative entre la présence de la végétation native (y compris le *C. ladanifer*) et le taux de survie des arbres et arbustes, ce qui indique que la végétation native peut leur faire concurrence. Cependant, il est plus probable que les arbres et arbustes plantés aient mieux survécu grâce à l'effet positif des amendements organiques, qui ont simultanément réduit la couverture végétale. Enfin, une régression linéaire entre la biomasse et le GVC a montré que le GVC est un bon proxy pour l'estimation de la biomasse.

1. INTRODUCTION

1.1. Contextualization

Climate change is undoubtedly threatening ecosystems, especially in the Mediterranean region, where drought and reduced precipitation, along with higher temperatures, are already widespread (Rama et al., 2022). In this particular region, the Mediterranean had been used to fires and has vegetation highly adapted to post-fire recovery (Rundel et al., 2018). The Mediterranean region, which is considered a biodiversity hotspot, is highly affected by alterations in the fire frequency and the projected extension of the fire season. These changes may lead to changes in plant post-fire recovery, particularly considering off-season fires, as climatic conditions may be difficult for plant recovery (Mateus & Fernandes, 2014; Rundel et al., 2018; Turco et al., 2019). Together with the vegetation and soil losses attributed to fires, this is a topic that requires great attention. Indeed, the United Nations established the settled The Global Goals in order to commit the world leaders towards sustainability. As wildfires are one of the main perturbations leading to soil degradation and ecosystem imbalance, there is an increasing need for efficient amendments, being one of the priorities of the 15th Sustainable Development Goal, titled Life on Land, which aims to "restore degraded soils by striving and achieving a land degradation-neutral world" (United Nations, 2016).

In this sense, and considering (i) the alteration on fire regimes and (ii) the emergence for soil recovery strategies, Portugal can be an interesting country to start with, as it is located in this Mediterranean region and is one of the top 10 countries with the largest forested area in the European Union.

1.2. Wildfires in Portugal

Wildfires have increased in intensity and extent in Portugal over the past decade, which has been facing a high number of fires and a large percentage of burned areas (Mateus & Fernandes, 2014; Nunes, 2012; Turco et al., 2019). The country is part of the Mediterranean ecosystem, where fire is normally "a significant ecological disturbance and regenerative process" (Nunes, 2012). However, in recent decades, such events have increased in intensity and magnitude and have lost their ecological role, becoming a highly relevant environmental problem in the area (Larchevêque et al., 2005; Nunes, 2012)

The increase in the frequency and extent of forest fires is the result of several climatic, geographic, and socioeconomic factors. The Mediterranean climate is known for its long, dry, and windy summers that favor forest fires, as well as for the high concentration of precipitation in autumn, which increases biomass fuel during the dry season (De Luís et al., 2001; Larchevêque et al., 2005; Nunes, 2012). Also, it is important to consider climate change scenario with increasing frequency of fire events (Turco et al., 2019) due to the rise in temperatures and a substantial reduction of the rainy season. The country also faces a decline in traditional agriculture, a decrease in biomass demand for domestic and industrial use, leading to more compact forest and shrub masses on abandoned agricultural lands where fires can easily spread (De Luís et al., 2001; Nunes, 2012). High population density is also a factor that favors fire outbreaks (Nunes, 2012), as it increases the number of human-caused ignitions.

Wildfires can greatly alter ecosystems and affect soil structure and properties, contributing in large part to land degradation and desertification (Nunes, 2012). For example, they consume some of the plant material and litter, as well as organic matter in the upper soil layers (Guerrero et al., 2001). Such events can also affect the microbiota in the soil and the vegetation cover, leaving the soil unprotected against the impact of raindrops (De Luís et al., 2001). This favors erosion processes associated with higher nutrient and soil losses, with negative effects on soil and forest vegetation recovery. Human intervention is needed to accelerate forest recovery, and reforestation is a common approach to achieve this (Gomez-Aparicio et al., 2004)

First, strategies must be implemented in Portugal to prevent forest fires (including prevention, land management, land occupation). Then, strategies must be considered to help burned ecosystems to regenerate faster and properly. This work is part of this goal and explores practices that can be applied to burned lands to help reforestation and restoration.

1.3. Soil treatments on burnt areas and their potential effect on soil, native vegetation, and seedlings

In Mediterranean areas, reforestation is associated with extremely high plant mortality rates (Gomez-Aparicio et al., 2004). Therefore, human intervention is often needed to support secondary succession to shrub or even grass level and to accelerate forest recovery of woodlands.

Improving soil parameters is essential for successful seedling survival as well as the regeneration of native vegetation, and thus decreasing damages caused to the soil by forest fires (Pouyat et al., 2020, pp. 145–160). Native vegetation regrowth can indeed help the regeneration of ecosystems burned by wildfires as it protects the soil against erosion processes (Guerrero et al., 2001) and degradation (Albaladejo et al., 1998). Soil organic matter (SOM), which will be particularly addressed in this work, is an essential indicator of soil health that contributes to soil fertility in many ways, including increasing water-holding capacity and nutrients available for plants to grow (Wilson, 1991). It also enhances both soil structure which reduces erosion and allows better access for roots to nutrients; and plant carbon sequestration which increases plant growth (Pouyat et al., 2020, p. 32). Soil moisture was thus also investigated as it allows better plant growth.

Various soil treatments have been shown to be beneficial for both soil and vegetation in several studies: (1) creation of contour bunds; (2) application of a mulch layer of woodchips; (3) addition of compost. The purpose of this work is to, in one way investigate whether these treatments increase tree and shrub seedling survival and, on the other way, increase biomass of native vegetation.

First, (1) creating contour bunds on the field has been shown to be effective in increasing soil infiltration rates and reducing runoff, thereby reducing erosion (de Figueiredo et al., 2012). Second (2), the addition of a mulch layer of woodchips can be very effective in reducing post-fire losses (S. Prats et al., 2014,2019). This addition of organic material (OM) enriched in macro- and micronutrients can also aid recovery and shorten the time needed to achieve an adequate level of soil protection. Third (3), numerous authors claim that the addition of a compost layer promotes the development of trees and shrubs (Guerrero et al., 2001; Larchevêque et al., 2006; S. Prats et al., 2014). The use of compost can also promote the growth

of native plants (Martínez et al., 2003). Indeed, high organic matter content can "improve the physical, chemical, and biological properties of the soil and consequently accelerate plant establishment."

Monitoring native vegetation may also be necessary for the success of planted species' seedlings. Some studies have shown that the success of planted seedlings was negatively affected by the presence of other species due to competition for light, nutrients, or water (Bohlman et al., 2016). In contrast, other authors (Castro et al., 2002; Gomez-Aparicio et al., 2004; Larchevêque et al., 2006) have shown that vegetation helps to retain water in the soil and thus could favor seedling survival. One purpose of this study will be to clarify whether or not native vegetation affects seedling survival rate.

1.4. Context of the study within R3forest project

This work is part of a larger research project called "R3forest - Using exotic biomass for post-fire recovery - Reuse, Regenerate and Reforest". Its main objective is to " implement and evaluate an array of technical solutions simultaneously, which use best practice synergies to achieve a healthy soil, tackle erosion, increase biodiversity, manage invasive species and maintain forest production at the same time" (R3forest, 2021).

Therefore, several treatments (precisely explained in the next section "Materials and methods") were conducted on a burned soil to test their effects on the above R3forest objectives, including the plantation of trees and shrubs seedings. This work is focused on (i) the impact of the different treatments on the regeneration of native vegetation and (ii) the impact of the different treatments on the planted trees and shrubs seedlings as part of the reforestation effort.

The term "native vegetation" is used to talk about vegetation that regenerates on its own in the field and thus is not voluntarily induced. The decision to call the vegetation "native" was made because indigenous plants from the area are dominant in the field (Sergio Chozas, personal communication).

1.5. *Cistus ladanifer* as an indicator species

In addition, this study investigates a particular species, namely *Cistus ladanifer* (*C. ladanifer*), a dominant obligate seeder shrub species endemic to the Mediterranean region (Frazão et al., 2018). This species was chosen because it was the most abundant native species in the field. Several authors claimed that its presence can trigger competition for water and nutrients and inhibit the growth of several other plants by releasing allelochemicals (Bohlman et al., 2016; Frazão et al., 2018). Other authors claimed that this species can also act as a "nurse plant" and "plays an important role in water-limited systems in controlling erosion, maintaining biodiversity, promoting plant growth-promoting rhizobacteria, and facilitating tree regeneration" (Castro et al., 2002; Ramos Solano et al., 2007a; Ruiz-Peinado et al., 2013). The presence of *C.ladanifer* can also be seen as high biological interest because it quickly establishes in poor or burned soils, limits erosion, maintains biodiversity, and contributes to carbon sequestration (Silva Dias et al., 2019).

1.6. Aim of the study

The main objective of the study is to evaluate the effects of the treatments on native vegetation, shrub and tree seedlings and to examine the interaction between them. The research will focus on (**1**) the influence of soil treatments on soil properties; (**2**) the influence of soil treatments on native vegetation (including *C. ladanifer*) and interactions with soil properties; (**2bis**) the development of a simple field biomass assessment tool using green vegetation cover as a proxy; (**3**) the interactions between the presence and development of native vegetation (including *C. ladanifer* as a potential indicator species) on the survival rate of tree and shrub seedlings planted in the project.

For the first specific objective (**1**), this study will test the variables of soil moisture (SM), soil organic matter (SOM), and stones greater than 2 mm in diameter (ST). The latter was examined to determine if the proportion of stones differed between plots and if treatments could affect the proportion of rocks in the soil.

For the second specific objective (**2**), green vegetation cover (GVC), plant moisture (PM), dry biomass (DB), and wet biomass (WB) variables will be tested for native vegetation in general. A sub-objective (**2bis**) regarding native vegetation concerns the creation of a simple tool to assess biomass in the field. This parameter is indeed important to represent plant growth and carbon assimilation, but it is an expensive and time-consuming variable to collect in the field (Lati et al., 2011). Therefore, both GVC and biomass will be collected to see if GVC could be a good proxy for explaining DB and thus not having to collect it in the field.

The variables tested for *C. ladanifer* are plant height (Cistus H), new growth height (Cistus NGH), old growth height (Cistus OGH), dry weight (Cistus DW), plant abundance (Cistus PA), stem diameter (Cistus SD), number of branches (Cistus NrB), leaf area (Cistus LA), leaf specific area (LSA) and plant moisture (Cistus M).

As will be explained in the next section "Materials and Methods," this study is particularly interested in the influence of contour bunds for the (**1**) soil and (**2**) native vegetation parameters studied. Indeed, a field observation showed a visual difference between the vegetation cover inside the swales (called on swales (OS) in this work) or on the ridges (called between swales (BS) in this work). It is therefore interesting to investigate whether or not these visual differences can be proved and if other parameters are affected.

The third specific objective (**3**) will investigate the potential influences of the previously mentioned variables on the survival rate of tree and shrub seedlings planted in the field. First analyses will specifically examine whether cover and the presence of native vegetation influence survival rates. Then, other analyses will investigate whether *C. ladanifer* might be a relevant indicator species to evaluate the effects of treatments on native species recovery and/or predict the success rate of planted tree and shrub species.

A table listing the name and unit of all the variables previously mentioned can be found in Appendix 1.

1.7. Perspectives for landowners

This work could also help landowners to seek best management practices after a wildfire. Depending on what purpose they see for their land (agriculture, forest, pasture, fallow, etc.) landowner may indeed look for different kind of landcover. Some may seek abundant and humid vegetation for their livestock, others may want to regenerate their soil first, or still others may want to rebuild the ecosystem through reforestation. The results of this work could thus help stakeholders in managing their land.

Since *C. ladanifer* is being studied as an indicator species, this could also be a way for landowners to monitor their land and get an indication of soil richness. It can also be used as a dietary supplement for ruminants when pasture is scarce, especially in summer (Dentinho et al., 2005; Guerreiro et al., 2016).

2. MATERIALS AND METHODS

2.1. Study site implementation

2.1.1. Area

The study site is situated close to Barão de São João, Algarve, in the south of mainland Portugal (37.14200382288447, -8.81195645695701), on the slope of a hill (10-15°), east-facing and with an elevation varying from 150 m to 180 m. The site was burned by a forest fire that consumed 2200 ha in the region in June 2020. The site is part of an ecosystem with a hot-summer Mediterranean climate (Csa, Köppen climate classification), characterized by hot and dry summers and warm and more humid winters, as well as sclerophyllous vegetation (Beck et al., 2018; FAO, 2015). The area has two soil types: chromic and ferric luvisols, consisting of shale, schist, conglomerate, marl, shale, dolerite, sandstone and limestone. Both soil types are stony soils. In the field considered by this work, soil was estimated as silty-sandy soil by Sofia Uyttendaele using a method developed by (Mwendwa et al., 2020) to classify soils in the field.

2.1.2. Plots and treatments

The field site consists of 35 plots of 100 m² (10m x 10m) each of which 5 different operations were applied. Except for 3 control plots, a bulldozer (KOMAT'SU D65EX-18, California, US) was driven across the field with a blade to remove pre-existing burned and regrown native vegetation in October 2020. This treatment was termed "Machine." This same engine reworked the plots (except for 8 Machine plots) with a ripper to create small contour bunds and swales every 2 meters, thus creating 5 contour bunds per plot. Subsequently, the swales of the contour bunds were either left uncovered ("Swales"), filled with woodchips ("Swales + woodchips"),
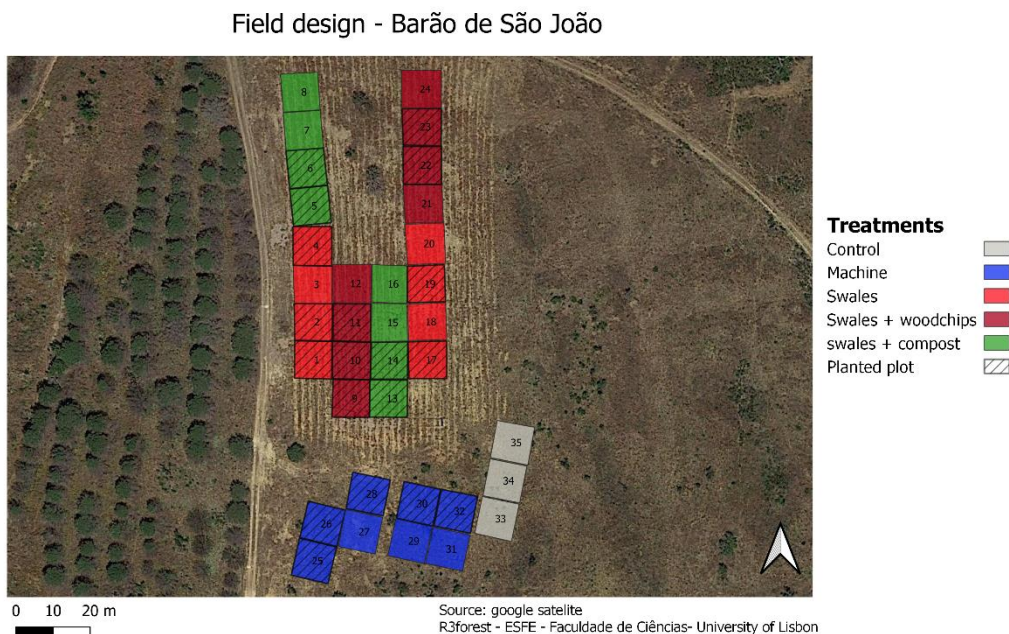
Figure 1: **Map of the field**. Plot's position with the information about the treatments applied and the plots where seedlings of trees and shrubs were planted.

or filled with compost ("Swales + compost"), with 8 plots for each. The application of compost and woodchip was carried out using a tractor equipped with a dispersion system (Polycrok 1750, Kuhn, Saverne, France) and took place in January 2021.

The 5 treatments are thus "Control", "Machine", "Swales', "Swales + woodchips" and "Swales + compost" and are shown in Figure 1. For convenience, treatments are simply indicated in this script as "Control", "Machine", "Swales", "Woodchips" and "Compost" when it is necessary.

2.1.3. Influence of contour bunds

The constitution of the plots with swales and bunds implies a potential difference in soil and vegetation's composition, depending on whether we sample on the swales (OS) or between these swales (BS). For this reason, for the plots concerned (Swales, Swales + woodchips and Swales + compost), samplings were carried out OS and BS. The representation of these specific areas is shown in Figure 2.
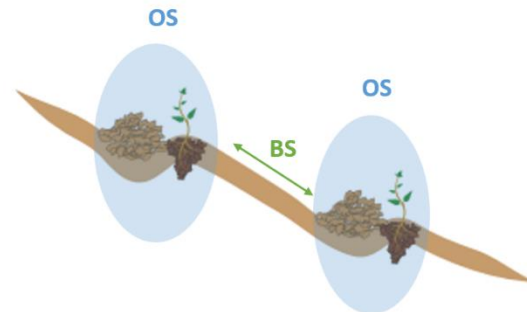
Figure 2: **Graphic representation of contour bunds**. Representation of the plot's areas on swales (OS) and between swales (BS). Original representation (R3forest, 2021) modified for the purpose of this study.

2.1.4. Planted tree and shrub species

On the 35 plots, 19 were planted with 8 different species: cork oak (*Quercus suber*), Eucalyptus (*Eucalyptus globulus*), rosmary (*Rosmarinus officinalis*), strawberry tree (*Arbutus unedo*), true myrtle (*Myrtus communis*), carob (*Ceratonia siliqua*), stone pine (*Pinus pinea)* and cypress (*Cupressus sempervirens*) and 14 were left blank to observe natural regeneration. The planted experimental setup is shown in figure 1. The plants were planted on the swales filled with compost and woodchips (Compost and Woodchips), in the swales left uncovered (Swales) or on bare soil without bunds (Machine). These planting areas are therefore located on the swales, as shown in Figure 2. The plantation took place in January 2021.

2.1.5. Compost and woodchips process

The compost was produced from chipped *Acacia longifolia* biomass with the help of a local agricultural company (Sousa Prado & Filhos, Agropecuária Lda, Mila Nova de Milfontes, Portugal), which is a partner of the R3forest project, and transported to the study site in Barão de São João. An area of ca 1 ha covered by *A. longifolia* trees was cut and the whole biomass was shredded into chips. A pile was formed, left in place and moistened for one year, during which time composting was carried out[1]. The woodchips were created from *A. longifolia* and *Pinus spp.* trunks and the piles formed were kept protected from water.

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[1] To ensure that the composting process degraded acacia seeds, germination tests were conducted to prove the efficiency of the process, which proved to be correct, as none of the tested seeds germinated. In addition to the heat generated by the composting process, it is the presence of fungi, favored by the humidity of the process, that destroys the acacia seeds (Joana Jesus, personal communication).

2.2. Sampling design

Sampling took place between 13-16 April 2022. Vegetation cover assessment as well as biomass and soil sampling were undertaken within each plot with an attention to contour bunds influence.

For plots where swales were created, the sampling methodology was applied OS and BS to test for a possible influence of the field set up on soil and native vegetation. For these treatments, six sampling points were randomly determined within the plots, of which 3 points were located OS and 3 points BS, as shown in Figure 3. For Control plots, only 3 points were sampled. Soil sampling, vegetation harvesting, and photography were all taken from these same sampling points.

As *C. ladanifer* was used as an overall indicator species of treatment effect, sampling was carried out without attention to the potential effect of contour bunds and 3 plants were randomly sampled in the entire plot.

Figure 3: **Single plot and sampling design**. Squares (58cm x 58cm) were randomly disposed in the plot. Blue squares are on the swales (OS) and green squares are between the swales (BL). Boxes are for illustrative purposes only. Original representation (R3forest, 2021) modified for the purpose of this study.

2.3. Green vegetation cover assessment

For each sample, a 0.34 m² (58 cm x 58 cm) quadrat was positioned over native vegetation or soil so that it surrounded randomized point. If the object was not exactly between or on a line, it was moved perpendicular to the boundaries of the plot until it was in the desired area. A picture was taken vertically at a height of approximately 1.20 m (the height of an outstretched arm) above the ground. In total, 177 photos were taken (6 per plot with contour bunds and 3 per plot without contour bunds). The 177 images were then processed using Photoshop software (Photoshop, Adobe, San José, USA) and cropped using the "Crop by Perspective" tool to preserve only the area within the frame. The final step was to analyze them using the Canopeo web application (2022 Canopeo App, Oklahoma State University Department of Plant and Soil Sciences, Oklahoma City, USA) which generates a percentage of green vegetation cover (GVC) for each image by classifying the image into white (green vegetation) and black (non-green vegetation), as shown in Figure 4.

Canopeo analyses images with an automatic color threshold classification, using colors in the red-green-blue (RGB) system. All the pixels in the images are analyzed according to ratios of R/G, B/G and the excess green index, resulting in a binary image where white pixels correspond to green canopy and black pixel to not green canopy (Chung et al., 2017; Patrignani & Ochsner, 2015).
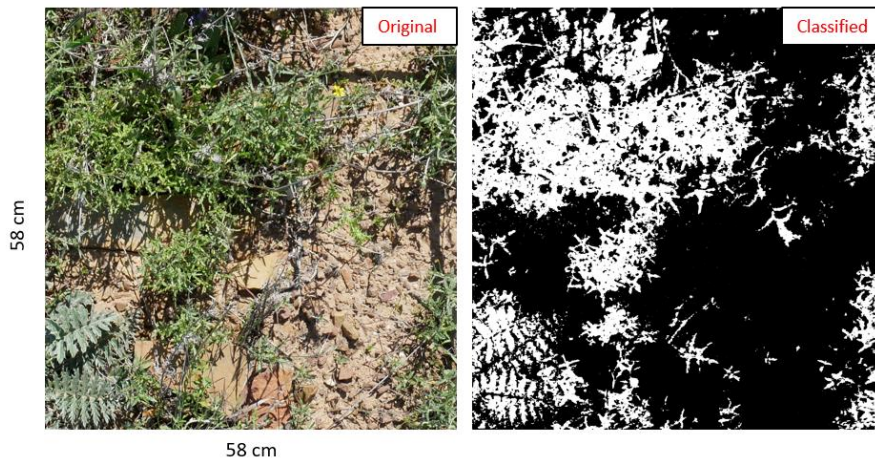
Figure 4: **Image processing with Canopeo to calculate GVC.** These figures show GVC pictures after cropping in Photoshop (original) and after classification by Canopeo (classified). Green vegetation is classified as white and non-green vegetation as black.

The percentages were averaged to obtain only two values per plot corresponding to the values of GVC for OS or BS.

2.4. Biomass collection and assessment

In this study, emphasis is placed on dry biomass because it is generally considered to be "an important absolute ecological and growth parameter representing carbon assimilation rate and plant development" (Donald, 2006 *in* (Lati et al., 2011).

Figure 5: **Frames used to collect GVC pictures and biomass.** The big frame (58 cm x 58 cm) was used to photograph vegetation and the little frame (20 cm x 20 cm) used to harvest biomass and collect soil.

Within the quadrat used for GVC, the upper left 400 cm² (20 cm x 20 cm) aboveground vegetation higher than 1 cm was harvested (Figure 5). Vegetation collected from the three different sampling points, BS and OS, was joined in airtight plastic bags to form two composite samples per plot. Samples were stored in a cool place until they were weighed to determine their wet weight (WW) (PGW3502e, AdamLab, Oxford, UK). They were then dried for two weeks at room temperature (20-25°C) in a drying room and for 5 days in a drying oven at 65°C to ensure complete water loss (Memmert, Schwabach, Germany). The dry weight was then determined, giving the dry biomass (DB) value (PGW3502e, AdamLab, Oxford, UK). Finally, the data were multiplied by 25 to obtain the dry and wet biomass for an area of 1 m².

The plant moisture (%) was calculated using the following formula:

$$PM\ (\%) = \left(\frac{WW - DB}{WW} \right) \times 100$$

2.5. Soil collection and analyses

Within the 400 cm² quadrat used for biomass harvesting (see Figure 5), a soil sample was collected with a small shovel (volume not measured). The soil collected from the three different sampling points for BS and OS was joined in an airtight plastic bag to form two composite samples.

Each sample was divided into two parts: One part (A) was dried at room temperature for two weeks and stored for possible further analysis, and the other part (B) was weighed to determine fresh weight (Soil FW) and then dried at 65 °C until constant weight. The samples (B) were then weighed to determine the dry weight (Soil DW), which is essential for calculating the soil moisture (SM):

$$SM\ (\%) = \left(\frac{Soil\ FW - Soil\ DW}{Soil\ FW} \right) \times 100$$

The samples (B) were then sieved using a 2 mm sieve. The subsample remaining in the sieve (over 2 mm) was weighed and discarded, providing information on the percentage of stones over 2 mm of diameter in the soil (ST).

$$ST(\%) = \left(\frac{Mass\ of\ soil\ over\ 2\ mm}{Soil\ DW} \right) \times 100$$

The subsample that passed through the sieve was weighed before being ground with a ball mill (Mixer mill MM 400, Retsch, Haan, Germany) to ensure its homogeneity. Soil organic matter (SOM) was assessed using a modified loss on ignition method (Heiri et al., 2001). For this method, 1 g was taken from each dry sample, weighed accurately (0.9800 – 1.0200) (AS 220.R2, Radwag, Radom, Poland), placed in a crucible, and burned in a muffle oven (L3, Nabertherm, Lilienthal, Germany) at 550 °C for 4 hours. This corresponds to soil before cooling (Soil BC). The sample was then weighed after cooling (Soil AC) to determine the soil organic matter, using this formula:

$$SOM\ (\%) = \left(\frac{Soil\ BC - Soil\ AC}{Soil\ BC} \right) \times 100$$

2.6. Data collection of *Cistus ladanifer*

In each plot, 3 plants taller than 15 cm were randomly selected and harvested. Plants were first measured from the highest point to the ground for *Cistus* **height** (Cistus H) and then cut at ground level. The three plants were placed in a plastic bag and stored in a cool place before the total sample was weighed for *Cistus* **wet weight** (PGW3502e, AdamLab, Oxford, UK) (Cistus WW).

The 3 plants were then treated individually for further analysis:

- **The *Cistus* new growth height** (Cistus NGH in cm) of the plant was measured with a meter. This corresponds to the green, non-lignified part of the stem.

- **The *Cistus* old growth height** (Cistus OGH in cm) represents the height of the plant last year and is calculated using the following formula:

$$Cistus\ OGH = Cistus\ H - Cistus\ NGH\ (cm)$$

- *Cistus* **stem diameter** (Cistus SD in cm): the thickness of the plant's stem was measured with a caliper (DIN 862, Wurth, Künzelsau, Germany).

- On each plant, the *Cistus* **number of branches** was counted in addition to the main stem (Cistus NrB in unit/plant).

- To calculate the *Cistus* **leaf area** (Cistus LA in mm²) of each plant, 4 leaves were randomly selected from each plant and placed on an A4 sheet. After covering the sheet with a Plexiglas plate to flatten the leaves, a picture of the leaf was taken from above. This image was then processed using Photoshop (Adobe, San José, USA) to crop the image while maintaining the proportions of an A4 sheet (210 mm by 297 mm), and to homogenize the background so that the image had only 12 units (12 cistus leaves). As shown in Figure 6, the color of the leaves was also changed to a uniform green color to calculate the leaf surface afterwards. To do so, the ImageJ software was used (Image J, National Center of Health, Bethesda, USA), a java-based free imaging program used primarily used in medical research (Abramoff et al., 2004). After specifying a pixel-cm measurement scale and selecting the desired leaves on the image, the ImageJ software calculated the leaf area for each plant (cm²). The software then created a csv file containing the leaf areas of the 105 sampled plants.

- For calculation of **specific leaf area** (Cistus SLA in mm²/mg), leaf samples used for determination of Cistus LA were kept in a plastic bag and dried at 65 °C for one week in a drying oven (Memmert, Schwabach, Germany) and weighed (AS 220.R2, Radwag, Radom, Poland) to obtain the leaf dry weight (Cistus LDW). The Cistus SLA was then calculated using the following formula:

$$Cistus\ SLA = \frac{Cistus\ LA}{Cistus\ LDW}\ (\frac{mm^2}{mg})$$

- *Cistus* **dry weight** (Cistus DW in g): the composite samples of 3 plants each were placed in the drying oven (Memmert, Schwabach, Germany) for one week at a temperature of 65°C as soon as possible after the field. They were then weighed (AS 220.R2, Radwag, Radom, Poland). The dry weight of the leaves was previously added to the final dry weight before the data were used.

- The *Cistus* **plant moisture** (Cistus M in %) was calculated using the following formula:

$$Cistus\ M = \left(1 - \frac{Cistus\ DW}{Cistus\ WW}\right) \times 100\ (\%)$$

- **Plant abundance** (Cistus PA in unit/plot) was assessed using pictures from GVC determination (see Figure 4). For each image, numbers of *C. ladanifer* were counted and mean to have an average number of plants per treatment.
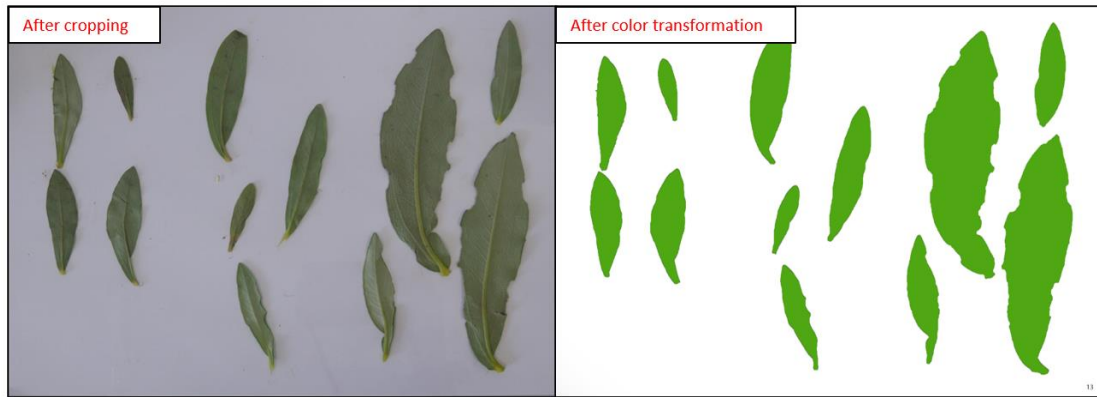
Figure 6: **Image processing in Photoshop for further utilization with ImageJ**. Image of leaves from 3 *C. ladanifer* plants harvested in one plot before (after cropping) and after transformation (after color transformation) with Photoshop. This transformation is needed to homogenize the colors and calculate precisely leaf area per plant with ImageJ. Leaves per plant are grouped by 4, which makes 12 leaves in total.

All variables were mean by plot, created a datafile of 11 variables with 1 value per plot (35 values).

2.7. Seedling survival rate

The survival rate of seedlings planted in the planted plots (visible in Figure 1) was measured in December 2021 by Florian Ulm. He visually certified the survival or dieback status of each planted tree and shrub. The csv file created from this sampling (Seedlings_Survival 2021) was used in this work. For each planted plot, the percentage of living trees relative to the number of planted trees was determined using Microsoft Excel software (Microsoft 365, Redmond, USA). A data file was created with 19 plots and their survival rates.

2.8. Statistical analysis

All statistical tests were performed using R 4.1.1 software and the Rstudio application (Rstudio, Boston, USA).

When the treatments in general were compared (without the influence of BS or OS), 35 data (one per plot) were used with this number of replicates: 3 for Control, 8 for Machine, 8 for Swales, 8 for Woodchips and 8 for Compost. When the treatments were analyzed with the influence of BS and OS, 59 data (2 per plots with contour bunds) were used with this number of replicates: 3 for Control, 8 for Machine, 16 for Swales, 16 for Woodchips and 16 for Compost.

All data used for analysis of variance (ANOVA) or T-test (pairwise-t-tests) were tested for homoscedasticity using a Brown-Forsythe Levene type test and for normality using a Shapiro-Wilks type test (Millot, 2014). In cases where the conditions were not met, those following tests were used according to the desired analyze:

- Kruskal-Wallis tests instead of an ANOVA test to detect a difference in means for at least one group,
- Pairwise-Wilcox tests instead of a pairwise-test to detect a difference in means between groups tested.

Normality of distribution was also checked using the Shapiro-Wilcox for the data to realize correlations. If the conditions were not met, Spearman correlations were performed instead of Pearson correlations.

2.8.1. Effect of treatments (soil and native vegetation)

First, a mean value per plot was calculated for all samples collected OS and BS i.e., SM, SOM, ST, DB, WB, GVC and PM.

Analysis of variance was conducted (ANOVA or Kruskal-Wallis) to determine if there was statistically significant difference between the treatments for the different variables tested.

If these tests confirmed a difference between the means of the different treatments, a pairwise-t-test or pairwise-Wilcox-test was performed to compare the means between the groups. These tests used in R allow to "calculate pairwise comparisons between group levels with corrections for multiple testing". The mention correction is Holm (RDocumentation, 2022).

2.8.2. Influence of the contour bunds (swales)

A nested ANOVA was conducted on the variables (SM, SOM, GVC, DB, WB and PM). This test allows to test the influence of a factor 1 "treatments" and to determine if a factor 2 "swales effect" is nested within factor 1, i.e. the possible influence of the sampling location in the plots (OS or BS) on the variables tested.

2.8.3. GVC to predict biomass

A model was created to determine if GVC (x) explained DB (y). Simple linear regression was used to estimate the parameters of the curve connecting the responsible variable (GVC) to the predictor variable (DB), and to test whether this relationship was significant. The independent variable was thus GVC and the dependent variable were DB.

First, the assumptions of linear regression were checked, i.e. linearity, homoscedasticity, uncorrelatedness, and normality (Peña & Slate, 2006; Poole & O'Farrell, 1971; Schmidt & Finan, 2018).

- Predicted linearity of the model was checked graphically using a scatter plot.
- Independence of the residuals was checked graphically using a lag plot and the Durbin-Watson test.
- Homogeneity was checked visually using a residuals vs fitted plot and a Breusch-Pagan test.
- The normality hypothesis was visually tested with QQplot and a Shapiro-Wilks test for the residuals.

If the conditions for model validation were not met, a series of transformations (log10, Ln, or square root) were applied to the explanatory variable (GVC) or to both explanatory and response variables (GVC and DB) until the assumptions were met as best as possible. These transformations are typically used when the data have positive skewness, and an exponential component is suspected in the data (IBM, 2020).

2.8.4. The influence of the treatments on Cistus Ladanifer

A principal component analysis (PCA) was performed to examine total variance of the data set. When the variables were by definition correlated because they were calculated from one another, a selection was made to keep only one variable. The remaining variables were: Cistus LA, Cistus LSA, Cistus DW, Cistus M, Cistus SD, Cistus NrB, Cistus PA, Cistus H, and Cistus NGH.

PCA was performed using the prcomp test in R with data's scaling and centering. A biplot was created to visualize how the data set was behaving.

To determine the influence of the treatment on the variables, pairwise comparisons (pairwise-t-test or pairwise-Wilcox-test) were conducted.

The variables for which treatments's influence could be attested were considered relevant to use in the next analyses (see next point 2.8.5.).

2.8.5. Interactions between native vegetation (including C. ladanifer) and survival of tree and shrub species.

After checking normality conditions and homoscedasticity, a pairwise-t-test or a pairwise-Wilcox-test was first performed to evaluate possible impact of treatments on survival rate values.

Then, a PCA was used to examine the interactions and correlations between the variables and plant survival rate. Finally, Pearson or Spearman correlation graphs were constructed between (i) seedling survival rate and native vegetation and between (ii) survival rates and Cistus-specific and relevant variables highlighted thanks to the previous analyses.

3. RESULTS

3.1. Treatment's influence on soil

All data conformed to normality and homogeneity, except for the normal distribution of SM values.

The Kruskal-Wallis-test performed on **SM** data set showed that at least one group (treatment) was statistically different from the others in terms of soil moisture (p-value < 0.05). The pairwise-Wilcox-test, on the other hand, showed no group differences.

The nested ANOVA applied to soil moisture content has shown that the factor 1 "treatments" has a statistically significant effect on water content in soils (p-value < 0.05) that the factor 2 "swale effect" does not and is thus not nested within the factor 1 (p-value > 0.05). However, as seen in Figure 7, there is a tendency for Compost and Woodchips to have wetter soil on the swales than between the swales, while the opposite is true for Swales (where the soil was opted bare).

The ANOVA test performed on the SOM data showed that one group was statistically different from the others (p value < 0.05). The difference between the treatments, without considering the influence of the swales, is shown in Appendix 2.

Both factors "treatments" and "swale effect" have a statistically significant effect on soil organic content. The nested test even shows a greater influence of the swales on the SOM values (p-value= 0.00489) than the treatments (p-value= 0.02779). Figure 7 shows this influence of the swales on the SOM values, which are higher on BS than on OS.

As the factor "swales effect" had an effect on the tested values (p-value < 0.05), a comparison of means (pairwise- t-test or pairwise- Wilcox- test) was performed by separating the data according to whether the sample was collected OS or BS, while keeping the machine and control values to test for differences with these treatments. The two datasets tested, resulting from splitting the original data set, were: Control, Machine, Swales BS, Woodchips BS, Compost BS (Sub-group BS) and Control, Machine, Swales OS, Woodchips OS, Compost OS (Sub-group OS), that are represented in Appendix 3. The conditions of normality and homoscedasticity were tested again for these subgroups.

The pairwise t-test applied showed that there was no statistical difference between SOM values for the subgroup BS. For the subgroup OS (where compost and woodchip are applied), the values for Swales, Woodchips and Compost are statistically different (and lower) from those of the control, as well as those of the machine compared to compost. In overall, SOM is higher in the control plot than in the other treatments in general[2] and is also higher BS than OS for plots treated with swales.

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[2] A pairwise-t-test was conducted on values without inclusion of swales influence (between "control," ,"machine", "swales", "swales + woodchips" and "swales + compost". It created groups according to the SOM results (see Appendix 1). Soil organic matter was statistically higher in the control plot, except with swales where no significant difference was found.

The ANOVA analysis of stones over 2 mm (ST) did not reject the null-hypothesis that all means were equal (p-value > 0.05). Neither the treatments nor the swales had a significant effect on the proportion of particles over 2 mm (p values > 0.05), ruling out this parameter as a relevant explanatory factor for the treatments on the soils.
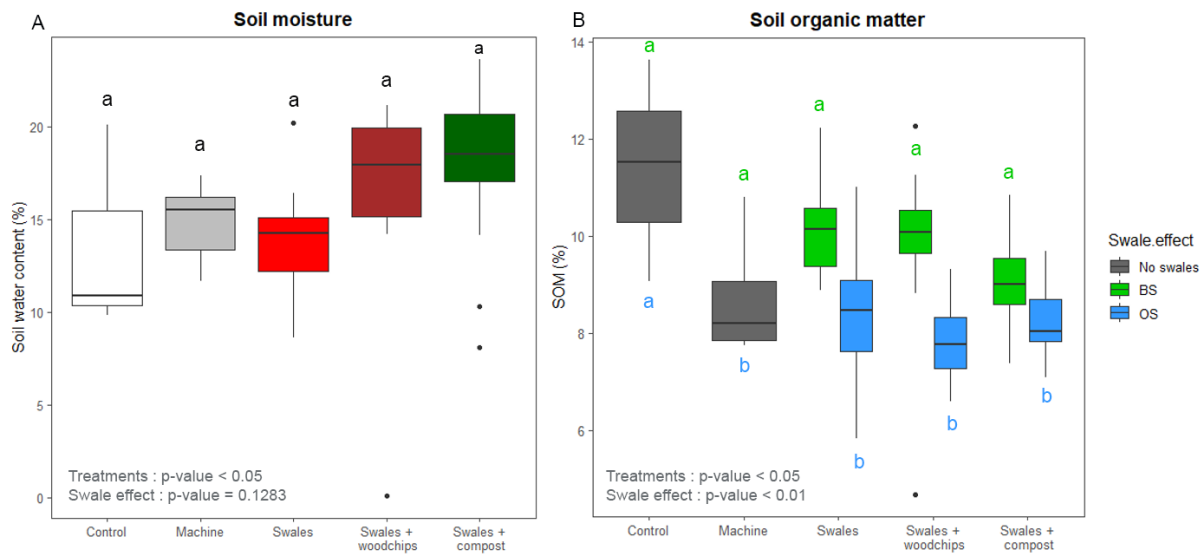
Figure 7: **Boxplots of SM and SOM by treatments and swale's effect**. P-values are the results of a nested ANOVA run on the dataset with an effect of the treatment or swales on PM and SOM values if p-values < 0.05. A pairwise-t-test was run on SOM values to assess the potential differences within the data for subgroup OS (written in blue) and subgroup BS (written in green).

3.2. Influence of the treatments on native vegetation

3.2.1. Influence on GVC, biomass and plant's moisture

Both GVC and PM fully conformed to the normal distribution and homogeneity of variances, but DB and WB data were not normally distributed (p-value < 0.05).

The use of Kruskal-Wallis on **dry and wet biomass** (DB and WB) data showed that at least one group was statistically different from another (p-value < 0.05). The pairwise-Wilcox-test did not show differences between treatments in terms of WB, and only between Machine and Compost for DB. A tendency for DB to decrease between Control and Compost, however, can be seen in Appendix 2 that shows variations for treatments in general.

WB is not affected by the factor "treatments" but only by the factor "swale effect" This variable was therefore plotted according to its position OS or BS to visualize the differences according to the factor "swale effect" thanks to a pairwise-t-test (Figure 8). The results showed that the numbers of WB are significantly less high when it grew on swales than between the swales or in Control and Machine plots.

Nested ANOVA showed that both the factors "treatments" and "swale effect" have a statistically significant effect on **DB** (p-values < 0.001). Further analyses (pairwise tests) were thus conducted between subgroups OS and OS, the same way as for SOM. Normality and heteroscedasticity were previously checked for the subgroups regarding the values of DB: values of the subgroup BS did respect homoscedasticity but not normality and were thus analyzed through a pairwise-Wilcox-test. Distinctions within sub-groups are represented in green for the subgroup BS and blue for the subgroup OS (Figure 8). For sub-group OS, DB

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significantly decreased in Swales, Woodchips, and Compost in comparison with Machine and Control. This is not the case for the sub-group BS.
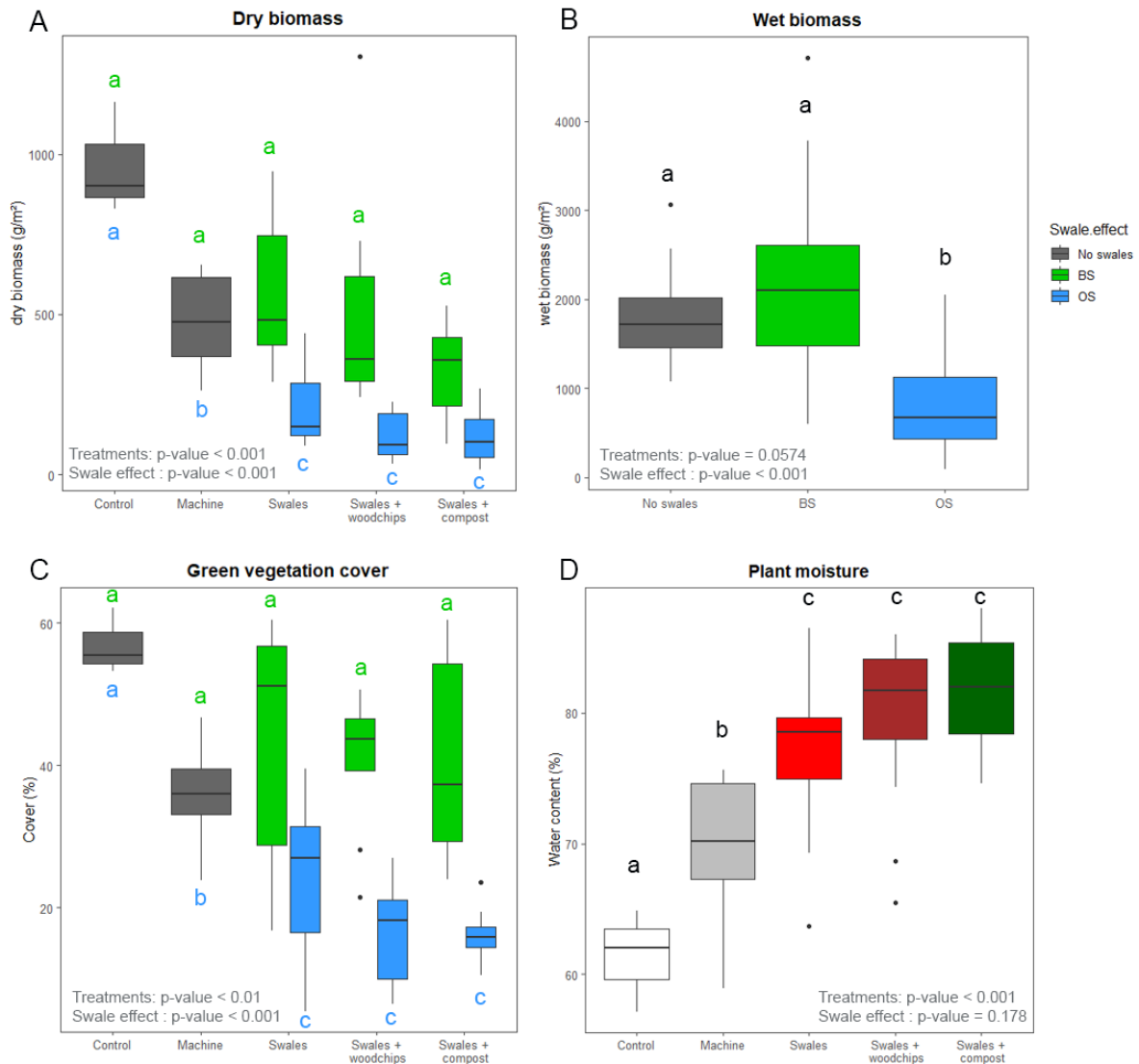
Figure 8: Boxplots of DB, WB, GVC, PM by treatments and swale's effect. P-values are the results of a nested ANOVA run on the dataset with an effect of the treatment or swales on data if p-values < 0.05. Pairwise-t-test or pairwise-Wilcox-test (only for DB of subgroup BL) was run on values to assess the potential differences within the subgroup OS (written in blue) and the data of subgroup BS (written in green).

Considering only treatment effect for **green vegetation cover (GVC)**, at least one group was significantly different from the others according to pairwise-t-test (p-value < 0.01). It showed the formation of a group A (Control) and a group B (Machine, Swales, Woodchips, Compost) where values are significantly higher for group B (Appendix 2).

Both the factors "treatments" and "swale effect" had a statistically significant effect on GVC. The Nested ANOVA used on GVC numbers even show a greater influence of "swales effect" (p-value < 0.001) than "treatments" (p-value < 0.01). Results of pairwise-t-test conducted for the sub-groups BS and OS are represented in Figure 8. As for DB, GVC is significantly less abundant in Swales, Woodchips, and Compost than in Machine and Control for sub-group OS but not for sub-group BS.

The pairwise-t-test run on **plant moisture** (PM) of harvested biomass showed the division of the dataset into three groups: A (Control), B (Machine) and C (Swales, Woodchips and Compost). The average moisture content of the plants in the control plots is 61.33% and ranges from 69.52% (Machine) to 81.89% (Compost) for other treatments. As this variable is not affected by the factor "swale effect" but only by the factor "treatments", the differences by groups highlighted are thus the same as the one represented in Figure 8.

3.2.2. Linear regression between biomass and green vegetation cover

After verifying the linear regression assumptions, it was necessary to modify the biomass and GVC values. Neither independence, normality, nor homogeneity of the residuals were present. The possible transformations listed in the "Materials and Methods" section have been applied to either the explanatory variable (GVC) only or to the explanatory and response variable (DB).

The log10 transformation applied to both DB (y) and GVC (x) values allowed maximum compliance with the conditions while maintaining positive intercept values. The only test invaliding the assumptions was the Breusch-Pagan test, which showed heteroscedasticity in the residuals (p-value < 0.05). However, the fitted plot in Figure 9 shows that the residuals tend to be uniformly distributed along the gradient of predicted biomass values.
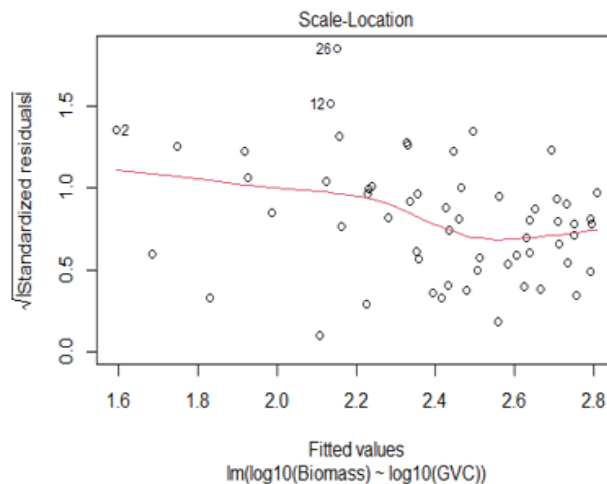
Figure 9: **Residuals vs fitted plot for linear regression between biomass and GVC**. The plot shows the fitted residue distribution for dry biomass (DB) values. The "fitted" correspond to the responses predicted by the model, for the observed values of the variable GVC. The red curve is the local regression curve.

In the statistical summary of the linear model, the p-value of the slope (< 0.05) indicates that there is a significant linear relationship between the response variable and the predictor variable.

A search for influential data was performed using a diagnostic plot (see Appendix 4) and revealed that item 26 was an outlier (confirmed by a p-value < 0.05 Bonferroni test). The final model was therefore constructed without this value, slightly changing the slope and intercept values. This outlier corresponded to a sampling point collected OS in a plot filled with compost, that showed exceptionally low values of biomass in comparison to GVC's values. Appendix 5 shows the 3 pictures that were used to calculate CVG for this composite sample as well as the areas that were cleared of vegetation to estimate biomass. These areas are particularly sparsely vegetated compared to the rest of the frame, giving particularly low biomass values compared

to GVC. This outlier corresponded thus to a problem inherent of the method and was removed to make sure it was not affecting the results of the analysis.

The final equation and representation of the model are shown in figure 10.

log (DB) = 0.8548 + 1.0868 * log (GVC)
(pval<0.001)
(R² = 0.55)
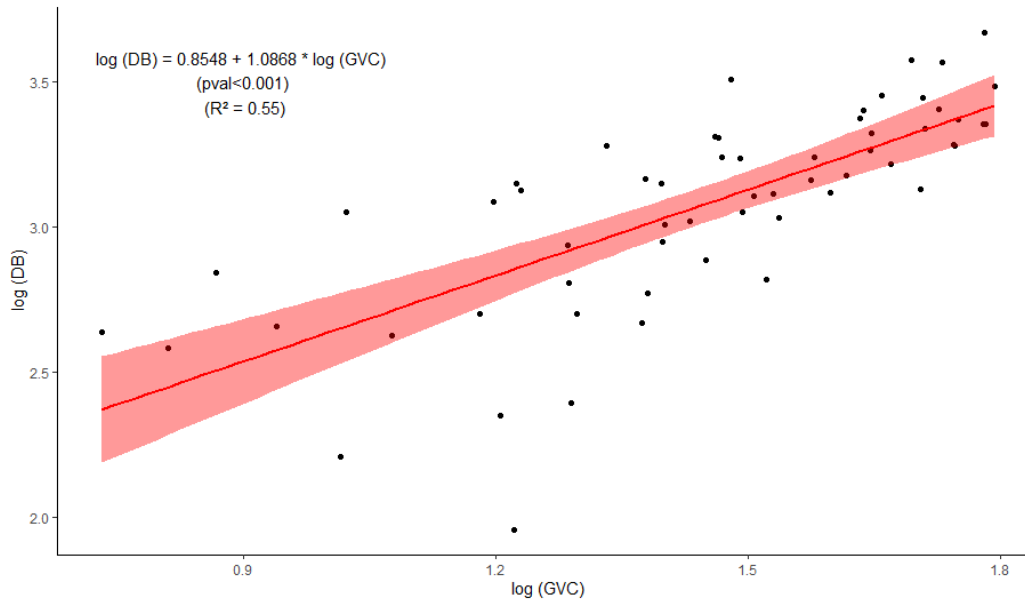
Figure 10: Linear model of dry biomass (DB) and green vegetation cover (GVC). The values of x and y have both been applied with a logarithm to the base 10 to respect linear model assumptions. The red line represents the linear regression and the red area corresponds to the 95% confidence interval of the slope.

The R² value of the model is 0.55, so 55% of the biomass values are explained by plant cover.

3.2.3. Cistus ladanifer as an indicator species

Behaviour of the dataset

After performing a PCA on the dataset, we obtained 9 principal components (PC1-9). Each of these components explains a certain percentage of the total variation in the dataset. PC1 explains 63.4% of the total variance, which means that 63.4% of the information of the dataset (9 variables) can be *encapsulated* by only this one principal component, and PC2 explains 19.2% of the variance. This means that if we know the position of a sample in relation to PC1 and PC2 only, we have an accurate idea of where it stands in relation to other samples and explains 82.6% of the variance. This is considered a high value in order to extract the most important information from the data with confidence using the interpretation of the first pair of aces (Borcard, 2018, pp. 125–126).

The biplot in Figure 11 summarizes the main features of the data set and show whether and how variables are organized into groups[3]. The lower left part of the biplot shows that plant abundance (PA) is highly, negatively correlated with all other variables, especially plant moisture (M), leaf area (LA), and new growth height (NGH). On the right side of the biplot, some variables are strongly correlated, such as height (H), stem diameter (SD) and number of

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[3] For reasons of comprehension and easier reading of the graph, the names of the variables have been abbreviated from their prefix "Cistus".

branches (NrB) and are mostly overlapping. Variables with nearly orthogonal arrows have correlation close to 0, such as several pairs in the cluster of variables in the right side of the graph i.e. leaf specific area (LSA) and dry weight (DW).

Control and Machine plots have the highest values for PA and the lowest values for the other variables, especially Cistus M and LSA. Compost, Woodchips and Swales have lower values of PA and form larger clusters covering the other variables, showing a broad distribution of the data.
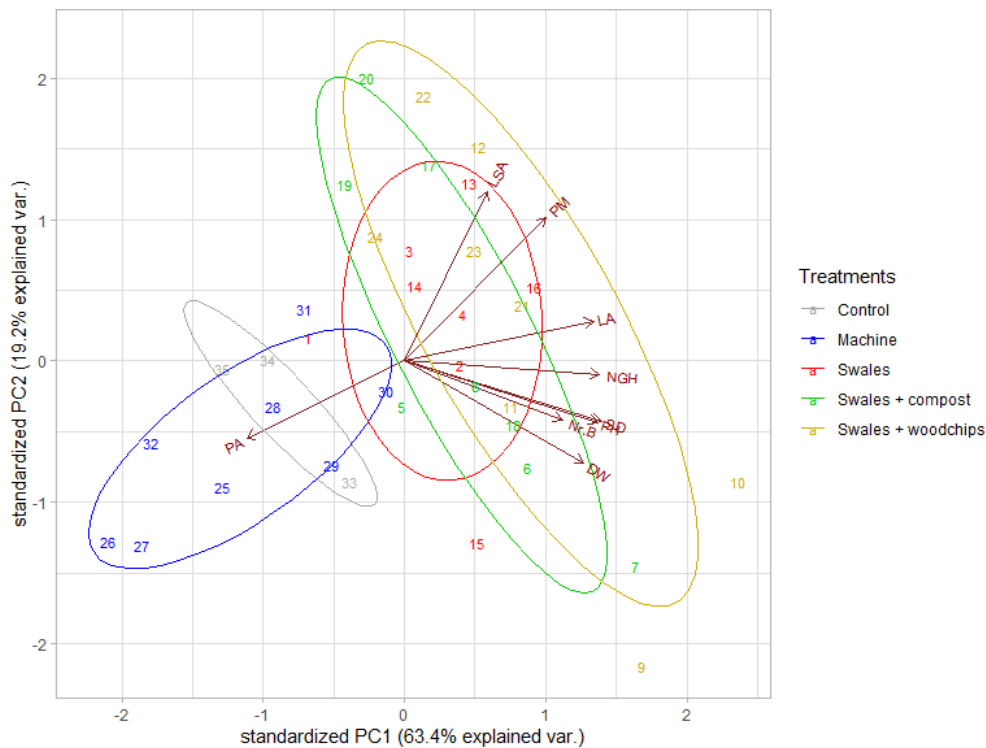
Figure 11: **Biplot of PC1 and PC2 from PCA run on dataset from *Cistus ladanifer*.** Data was scaled and centered, using the function prcomp in R. Variables are PA (abundance), LSA (leaf specific area), LA (leaf area), M (plant moisture), new growth height (NGH), stem diameter (SD), H (plant height), number of branches (NrB), dry weight (PDW).

The most important information from this biplot is that the dataset appears to be split into two clusters (Control and Machine VS Compost, Woodchips, and Swales), with PA as the most important explanatory variable. Strong correlations (negative or positive) also seem to exist between the variables.

A secondary analysis was undertaken to assess the correlations between PC1 and the variables. The graph in Appendix 6 highlighted that the variables H, NGH, SD, DW and LA are the most strongly and positively correlated with PC1 (R between 0.7 and 1) and that abundance is strongly and negatively correlated to this component. Those variables are therefore mainly responsible for PC1, that explains 63.4% of the variance.

Influence of the treatments on variables

Except for Cistus DW, the values of the variables were normally distributed, and all variances were homogeneous. The ANOVA tests showed that at least one of the means differed from at least one other in the target population for all variables (p-values < 0.05).

Depending on compliance with normality and homoscedasticity conditions, pairwise-t-tests or pairwise-Wilcox-tests were used for variables to separate the treatments into different groups (see Figure 12).
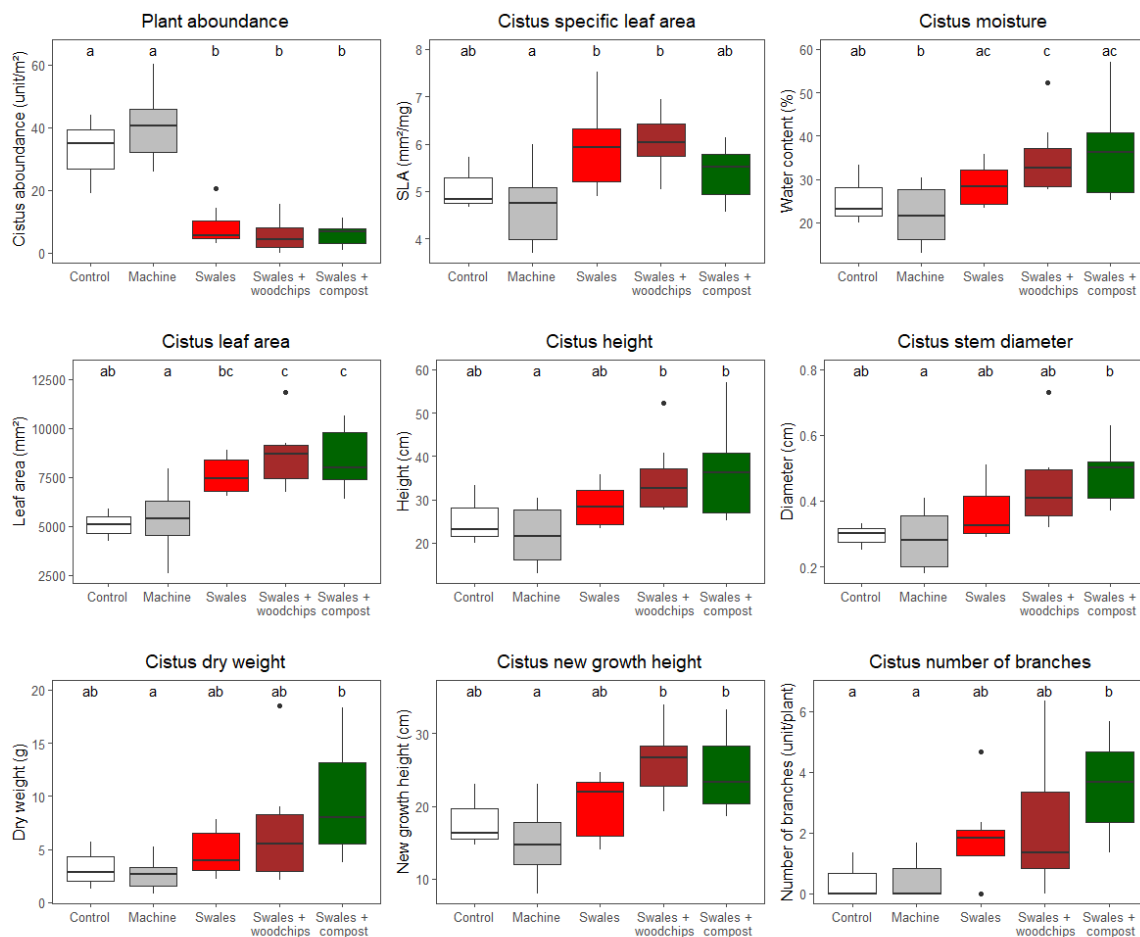
Figure 12: Boxplots of the *Cistus ladanifer*'s parameters by treatments. Variables were grouped by treatments and groups highlighted by pairwise-t-test (or pairwise Wilcox test for plant dry weight). Previous ANOVA and Kruskal-Wallis tests showed a difference in the treatment groups for all parameters.

As shown in Figure 12, the only parameter with a strong effect of treatments is PA, for which the pairwise-t-test confirmed the existence of two distinct groups: A (Control and Machine) and B (Swales, Woodchips, and Compost). For the remaining variables tested, the boxplots show the same tendency for the data to increase as we visualize the treatments (in order: Control, Machine, Swales, Woodchips, and Compost) with a demarcation between the first two and the last two treatments. Pairwise-t-test formed distinct groups with statistically different means for those variables, even if the demarcation between treatments is less obvious.

3.2.4. Interaction between soil and vegetation

<u>Biomass, GVC and soil parameters</u>

As the variables SOM, GVC, PM and DB showed a strong influence of treatments, correlations were performed using the Spearman method, since the values of DB did not follow a normal distribution (see point "3.2.1. Influence on GVC, biomass and plant's moisture").

The graph in Figure 13 shows a highly significant positive correlation between SOM and the presence of vegetation, with a coefficient of 0.54 for GVC and 0.46 for DB. There is also a negative correlation between the dry biomass and moisture content of vegetation.
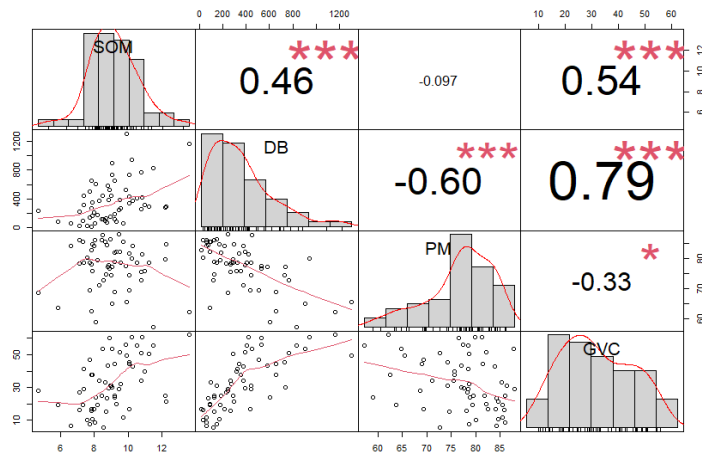
Figure 13: Correlation graphs between soil and native vegetation variables. Spearman correlations between soil organic matter (SOM), dry biomass (DB), wet biomass (WB), plant moisture (PM) and green vegetation cover (GVC). The correlation is using "spearman" method as values of DB are not normally distributed.

<u>*Cistus ladanifer*, native vegetation and soil</u>

No correlation (using Pearson method) was found between SOM values of the soil and the variables concerning *Cistus ladanifer*.

Nevertheless, a highly significant positive correlation was found between native vegetation's moisture (M) and *Cistus*'s moisture (Cistus M) as well as *Cistus*'s leaf area (Cistus LA), with coefficients of 0.57 and 0.59. A highly significant positively correlation was also observed between *Cistus* abundance (Cistus PA) and native vegetation's dry biomass (R = 0.56). On the other hand, a highly significant negatively correlation was found between Cistus PA and native vegetation's moisture (R= -0.71) as well as between Cistus LA and native vegetation DB (R = -0.51). The correlations are represented in Figure 17.

3.3. Influence on planted tree and shrub species

3.3.1. Treatment influence on seedling's survival rates

First, a pairwise-t-test of the survival rate (SR) of the planted tree and shrub species was performed to show the influence of treatments on this variable (see Figure 14). The test showed a statistically different and higher survival rate in the Compost plots than in the Machine and Swales plots.
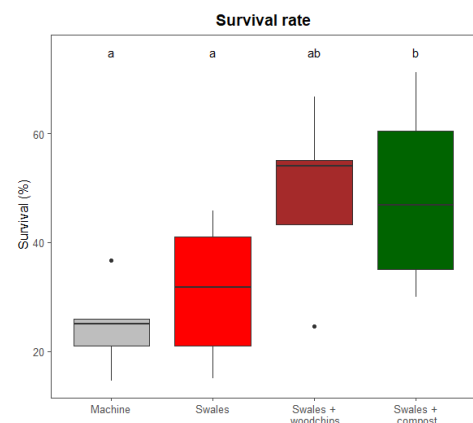
Figure 14: **Survival rate of seedlings by treatments**. SR were grouped by treatments and groups highlighted by pairwise-t-test. Samplings were collected in December 2021 on the plots concerned by tree and shrub seedlings plantation.

3.3.2. Influence of native vegetation on seedling survival rate.

<u>PCA</u>

The PCA in Figure 15.A shows the variation of the dataset of native vegetation. The first two principal components explain 81% of the variance of the dataset. GVC is strongly and negatively correlated with survival of planted tree and shrub species. On the biplot, the treatments seem to overlap, although some patterns can be seen: Machine treatment plots have more biomass, plants are more humid in compost and survival is higher in woodchip and compost treatments.

For *C. ladanifer*, 80.1 % of the total variance is explained by the first two principal components. The variable LA is strongly positively correlated with the survival rate of seedlings while PA is strongly and negatively correlated with the survival rate. All the other variables seem to be positively correlated with the survival rate.
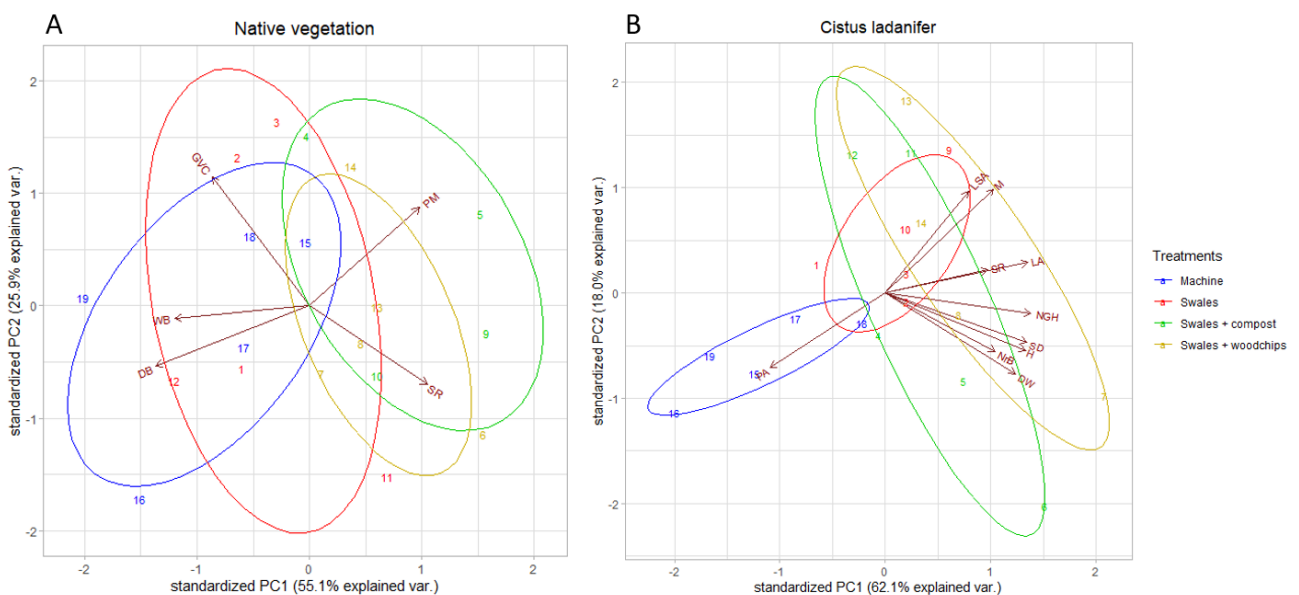
Figure 15: **Biplot of PC1 and PC2 from PCA run on dataset with native vegetation and survival rate (A) and with *Cistus ladanifer* and survival rates (B**). Native vegetation and *C. ladanifer* data set were used with survival rates, thus for 19 plots. Data was scaled and centered, using the function prcomp in R. Variables A: survival rate (SR), green vegetation cover (GVC), plant moisture (PM), wet biomass (WB), dry biomass (DB). Variables B: plant abundance (PA), leaf specific area (LSA), leaf area (LA), plant moisture (M), new growth height (NGH), stem diameter (SD), plant height (H), number of branches (NrB), dry weight (DW).

## Correlations

For native vegetation in general, the most significant correlation with SR is the one with GVC, highly significant and negative with a coefficient of -0.70 (see Figure 16). The correlation between SR and DB is also statistically negative but with a less strong relation (R = -0.41). Even if no correlations were proven by the test, it can be attested that the relationship between PM and SR is positive.
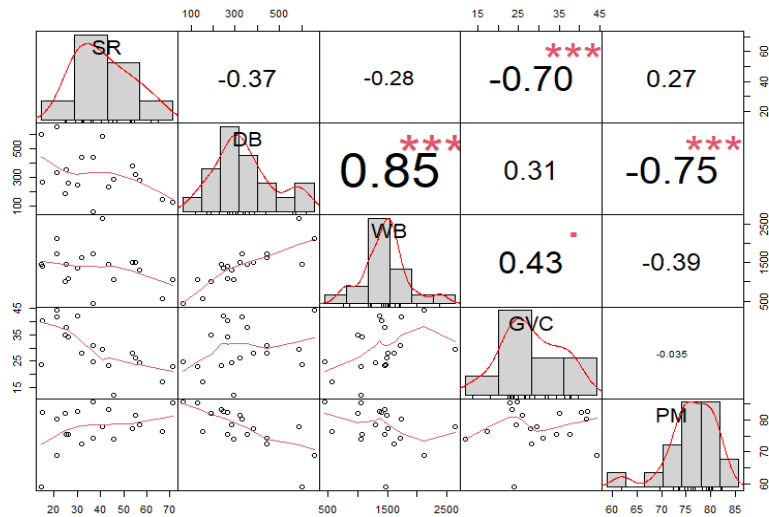
Figure 16: Correlation graphs between survival rate and native vegetation parameters. The correlations were realized using "Spearman" method as values of dry biomass are not normally distributed. The variables are survival rate of seedlings (SR), dry biomass (DB), wet biomass (WB), plant moisture (PM) and green vegetation cover (GVC).

Consistent with what the biplot (Figure 15) suggested, the correlation graph in Figure 17 shows that the values of LA had the strongest relationship with plant survival rates (R = 0.64). With the exception of plant abundance, all variables were positively correlated with survival rate values. Thus, the taller, wider, wetter, and broader are the leaves of the Cistus, the higher the seedling survival rate is. In contrast, the higher the abundance of Cistus is, the lower the survival rates are.
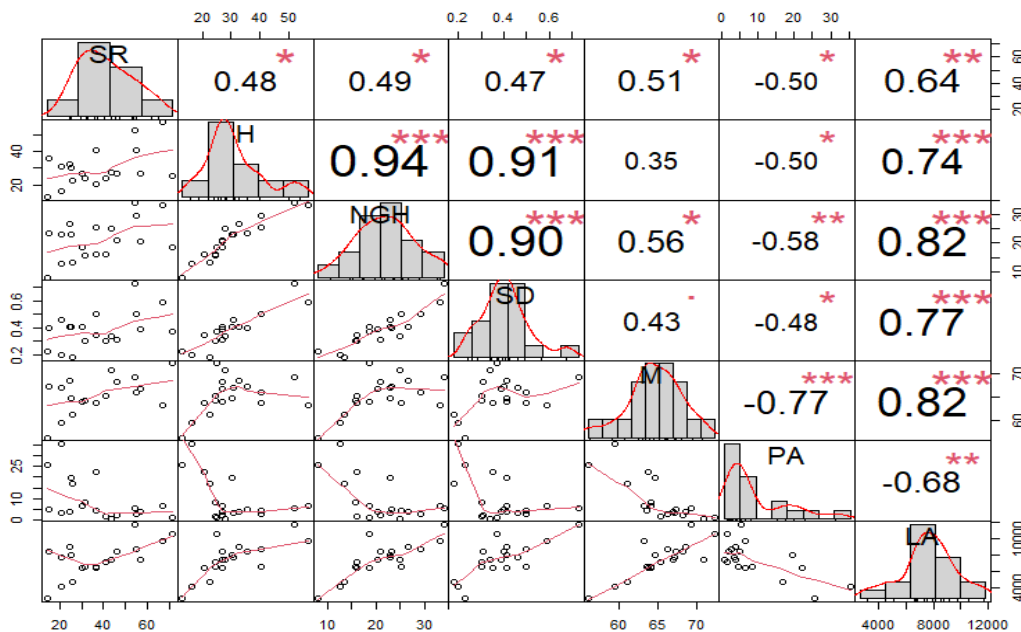
Figure 17: **Correlation graphs between survival rate of seedlings and *Cistus ladanifer*'s parameters**. The correlations were realized using the "Pearson" method all values follow normal distribution. The variables are survival rate of seedlings (SR), plant height (H), new growth height (NGH), stem diameter (SD), plant moisture (M), plant abundance (PA) and leaf area (LA).

4. DISCUSSION

The results will be evaluated and discussed in the same order as they were presented in the chapter 3 on results.

4.1. Treatments' influence on soil's properties

4.1.1. Water content

Contrary to expectations (Larchevêque et al., 2005), soil moisture did not vary as a function of the treatment applied (Figure 7). However, field samples were taken shortly after two days of rainfall, which could explain these statistically similar moisture contents. Thus, an additional pairwise-t-test analysis was conducted on soil moisture data (normally distributed) taken in December 2021 (after three weeks without rain) by Florian Ulm and processed in collaboration with Sofia Uyttendaele using the same laboratory methodology as the one described in "Materials and methods". Results are showed in Appendix 7. It showed that the soil in the Compost plots (Group B) was statistically wetter compared the Control, Machine, and Swales plots (Group A), and that woodchips plots were also wetter but not significantly different from Group A or B. This confirms that the soil seems to keep more moisture when a layer or organic amendment is covering the soil, which is in line with a study that showed organic amendments is indeed increase water holding capacity (Larchevêque et al., 2005).

Vegetation cover may also explain this phenomenon. Bohlman et al. (2016) showed that the presence of shrubs can lead to soil moisture depletion, especially when the environment faces water shortages. As it will be addressed in the next point (4.2.1) concerning treatments influence on native vegetation, there was indeed a higher vegetation cover and biomass in April 2022 (Figure 8) when the soil was less humid in December 2021 (Appendix 7). In contrast, other studies (Castro et al., 2002; Gomez-Aparicio et al., 2004; Larchevêque et al., 2006) have shown that vegetation helps to retain water in the soil. The results from the samples taken in December 2021 in relation to the one taken in April 2022 seem to overturn the findings of these authors and confirm the study of Bohlman et al. (2016). However, it is necessary to stay careful about these results as the sampling times are not the same. Further analysis should be undertaken when rainfalls do not affect the accuracy of the methodology.

Thus, if there is no indication that vegetation retains water in soils, it does so in its own biomass, that is more humid when contour bunds are created (Figure 8). This will be discussed in the below section 4.2.1 about plant cover and humidity.

4.1.2. Soil organic matter

Surprisingly, compared to previous results (Larchevêque et al., 2006; Prats et al., 2019), soil organic matter is lower in the treated plots (Swales, Woodchips and Compost) compared to the Control plots. Even more surprisingly, the organic content in the swales, where an organic amendment is applied (Compost and Woodchips), is even lower than between these (Figure 7).

There are some main reasons that could explain this phenomenon, namely vegetation cover (1), earthworks and stone cover (2), temporal effect of compost and woodchips admixture (3), and compost itself (4).

(1) Results showed that the more vegetated the plots, the higher the SOM values. Several studies (Albaladejo et al., 1998; Aranda & Oyonarte, 2005; De Luís et al., 2001; Guerrero et al., 2001; Martínez et al., 2003) show that the degradation or removal of vegetation cover negatively affects soil properties and decreases SOM. Plant cover can indeed reduce erosion and protects soils from degradation, avoiding a decrease in the organic matter stored in the soil and, consequently, a deterioration of its physical properties. Papers (Albaladejo et al., 1998; De Luís et al., 2001) have shown that fragile ecosystems, such as semiarid and arid areas, cannot fully recover after a severe disturbance, such as the complete removal of vegetation. Moreover, undeveloped vegetation, i.e., after prior removal, also leads to soil degradation (Aranda & Oyonarte, 2005). The results confirm this tendency (Figure and Appendix 7), since a sudden decrease in SOM values was observed once the plots are affected by vegetation removal (all treatments except Control). De Luís et al. (2001) added that when the vegetation cover is less than 30%, plants may no longer be able to retain soil particles, which concerns the ground OS that also face lower levels of organic matter. Thus, even if Prats et al. (2014) showed that soil cover, such as chopped wood or compost, is one of the most important key factors in mitigating soil erosion, vegetation cover seems to increase SOM the most.

(2) Machinery driving over the field may have removed organic matter residues and burned vegetation from the soil, which reduces the amount of organic matter. In addition, Prats et al. (2019) found that soil losses also depend on factors such as slope and rock cover. Since the terrain was observed to be heterogeneously rocky, this may directly influence the SOM results. Heavy machinery with ripping processing can increase rock fragment and reverse soil layers, and thus lower SOM. It does also significantly lower vegetation cover (de Figueiredo et al., 2012). Additional investigations could be implemented to attest to differences in rock proportions between plots (with a drone flight for example).

(3) Time could also affect SOM. Soil samples were collected one year and three months after compost and woodchips were added in the swales. Since the effects of organic amendments on soil decrease with time (Albaladejo et al., 1998; Guerrero et al., 2001), the effect could have already been completed. However, this period seems to be too short, and a study conducted by Martinez et al. (2003) even showed that it can take up to three years to observe a positive effect of biosolids application on soil organic content.

(4) A study conducted by Brito et al. (2015) showed that acacia compost has a lower organic matter content than most commercial substrates and recommended to use it with other organic materials that can increase the SOM. However, this study was carried out for horticultural purposes and substrates could not have the same effect on native vegetation in the field. Moreover, OM from compost could also degrade slowly (recalcitrance) and potentially not having affect the soil yet (Fabrizio et al., 2009). Thus, the compost might not have affected the soil organic matter.

4.2. Treatments' influence on native vegetation

4.2.1. Influence on native vegetation

Contrary to previous findings saying that organic amendment increases vegetation cover or plants health (Brito et al., 2015; Guerrero et al., 2001; Larchevêque et al., 2005, 2006; Martínez et al., 2003; Prats et al., 2019), the Control plots, where no treatments were applied, have significantly higher vegetation cover than the all the other plots (Figure 8).

The treatment consisting in removing native vegetation (Machine) seems thus to have the greatest effect on biomass and vegetation cover losses. A study (Larchevêque et al., 2005) also found lower vegetation cover in plots treated with compost than in control plots due to the unvoluntary but necessary squashing of vegetation by a machine. In their study, the effect of the machine disappeared after 6 months, but since their method (a tractor passing in the field to disperse compost) is less destructive than the one used in this study, the effects on vegetation are expected to last longer. Another study on soil preparation techniques for afforestation (de Figueiredo et al., 2012) showed that both treatments consisting in removing vegetation with a blame and passing in the field with a ripper to create contour bunds significantly lower vegetation cover. However, the plots where only vegetation was removed took three months to recover to their initial vegetation cover where plots with contour bunds did not reach their initial vegetation cover within the 2 years of the experiment. That could explain why in GVC is higher in Machine plots than in plots with contour bunds (Swales, Compost and Woodchips).

In addition, biomass and green vegetation cover did not increase with the application of compost and woodchips. In the plots where swales were created, biomass and vegetation cover OS were particularly low (Figure 8). This is likely due to the thick layer of compost and woodchips dispersed in the swale's ditches (OS), which inhibits the growth of native vegetation. This mulching effect was studied by Díaz et al. (2022) and Kruse et al. (2004), who found that if mulching had several beneficial effects on soils (diminution of runoff and erosion, increase infiltration rates), it lowers the vegetation cover. However, the same pattern is observed in plots with swales but without mulch application (Swales). Thus, in the same plots, vegetation cover is higher on ridges than inside swales, regardless of whether a litter is applied or not.

In contrast, plant moisture is higher in plots where Swales, Woodchips and Compost were applied than in plots treated with machinery or in Control plots (which are the less moist). When contour bunds were created, there were no statistical differences in plants collected inside the swales or on the ditches, i.e. between swales (Figure 8).
The vegetation being more humid can be explained by its abundance as less plants can lead to lower competition between species for water uptakes. Plants being moister could also indicate higher infiltration rates in plots with contour bunds (Díaz et al., 2022) that increase plant humidity. The creation of contour bunds also improves rooting depth (de Figueiredo et al., 2012), which could help plants growing more humid.
The differences in plant moisture between plots are also likely related to the fact that plants are younger where no vegetation removal was performed. This results in fewer mature woody plants, as evidenced by lower abundance of *C. ladanifer* (lignified species) (Figure 12). However, creating swales led to more humid vegetation than the one found in Machine plots, even if the vegetation was removed in both kind of treatments (Figure 8). The presence of more lignified vegetation in Machine plots can also be suspected because the abundance of *C.*

ladanifer was also higher (and equal to Control plots) than in plots with swales (Figure 12). The explanation cited above (higher infiltration rate and deeper rooting in contour bunds) can also justify the presence of less humid vegetation in Machine plots.

Soil water content, which was higher in the Woodchips and Compost plots 8 months earlier (Appendix 7), may also influence present-day plant moisture (Agee et al., 2002; Larchevêque et al., 2006; Mugnai et al., 2007).

These results show that creating swales and adding organic amendments help the ecosystem to be more resistant against future wildfire. Plant moisture is indeed a crucial characteristic to avoid future wildfire as foliar moisture content in shrubs and herbaceous vegetation act like a heat sink that lessens fire behavior (Agee et al., 2002).

4.2.2 Model between biomass and green vegetation cover

The model between biomass and GVC can be a great and simple tool to estimate biomass in the field as GVC is a proxy of the biomass. Indeed, 55% of the values of biomass are explained by green vegetation cover (Figure 10).

However, 45% of dry biomass values are explained by other parameters that were not considered in this model. According to previous studies (Axmanová et al., 2012; Lati et al., 2011; Ruiz-Peinado et al., 2013), the main additional independent variable should be the mean or median plant height. Axmanová et al. (2012) addressed the prediction of herbaceous biomass volume and found that the variables "total herbaceous layer cover" multiplied by "median vegetation stand height" were the best explanatory variables. In their study, the R² increased from 0.32 to 0.51 when this variable was added. Another model conducted by (Ruiz-Peinado et al., 2013) to estimate shrub carbon stock also used median height and canopy cover as explanatory variables. Thus, a good simple equation (which should be improved with data transformation if the assumptions of linear regression are not met) could be as follows:

$$Biomass\ (dry) = GVC * height\ (mean\ or\ median)$$

One advantage is that these variables are easier to collect in the field than the biomass itself. Axmanová et al. (2012) even suggested using the mean or median height of vegetation observed in the field from reference values, resulting in less work in the field. However, in degraded areas (such as the field from this study), this mean value may overestimate the actual mean value because the vegetation is relatively young.

One limitation is that the model could have been biased by the transformation of the variables. The transformation of the variables to reach the linear regression conditions indeed often leads to errors in the slope coefficient (Schmidt & Finan, 2018). Linear regression models are robust to the violation of the assumption of normality, in which case some authors (Poole & O'Farrell, 1971; Schmidt & Finan, 2018) claimed that data transformation is not necessary. However, linear models are less robust to the violation of homoscedasticity (Peña & Slate, 2006; Poole & O'Farrell, 1971; Schmidt & Finan, 2018).

In this study, several data transformation were tested before choosing to use a model with log addition on both dependent and independent variable. When no transformations were applied, neither the assumption of independence, normality or homogeneity were respected. This leaded to apply transformations on the dataset to respect more assumptions. The model that best fit was constructed by transforming both independent and dependent variables with logarithm, but

with which the assumption of homoscedasticity was still not met. However, a study conducted by Poole & O'Farrell (1971) showed that no precise test of homoscedasticity is possible as the tests existing, including the one used in this research (Breusch-Pagan test) are highly sensitive to non-normality of the data. The utilization of graphical test should be used to detect wrong assumptions (Schmidt & Finan, 2018) and the fitted-graph (Figure 9) was thus probably sufficient to meet the homoscedasticity.

Measurement error in the observation of the variables, and particularly the independent variable (GVC), also leads to biased estimated of the regression coefficients (Poole & O'Farrell, 1971). Some limits of the methodology used could be addressed and probably improved:

- GVC is estimated in a square of 0.34 m² and biomass is harvested in a square of 0.04 m². The vegetation used for biomass is less than 1/10 of the vegetation photographed to measure GVC. This may result in the harvested biomass not representing the actual vegetation present at the sampling point (as described in Appendix 5).

- When classifying pixels as green or non-green vegetation, flowers were excluded from the vegetation cover but were harvested for biomass.

- Compared to other refence image analyzers (Patrignani & Ochsner, 2015), Canopeo correctly classified 90% of the pixels, especially when the height and orientation of the camera were not correctly observed. For technical reasons, the camera was handled manually in the field, resulting in variable orientation and heights. However, the camera was always used by the same person at an average height suggested by the creators of Canopeo, i.e. 1,5 m (Patrignani & Ochsner, 2015). The images were also cropped in Photoshop to improve perspective, which reduces the uncertainty associated with this methodological bias.

Despite these statistical and methodological limits, the relationship between GVC and biomass is highly significant and the R² value can be considered good since no other explanatory variables were used. In addition, the use of Canopeo software allows for quick and inexpensive estimation of vegetation cover in the field without the need for spatial imagery or drone flight. Using the mean or median value of vegetation height (by using a field methodology or by using mean or median values of species in reference values) would improve the model without investing much more time and money in research.

4.2.3. Cistus ladanifer

The results show that *C. ladanifer* is less abundant in plots treated with Swales, Woodchips and Compost than in the Machine and Control plots (Figure 12). In contrast to native vegetation in general, *C. ladanifer* abundance is as high where the machine removed vegetation as in the Control plot. On the contrary, the presence of *C. ladanifer* is strongly inhibited when swales are created. However, as for native vegetation in general, the phenological parameters of the plants grown in the plots with swales (and even more with organic material amendment) showed better results. *C .ladanifer* in these plots tended to be larger, wetter, having wider bases, more branches and bigger leaves. Since *C. ladanifer* abundance is negatively correlated with all phenological parameters, and particularly with *C. ladanifer* moisture and leaf area, it confirms that where there are fewer individuals, the plants grow taller, moister, and with larger leaves (Figure 11 and Figure 17).

Differences in *C. ladanifer* abundance between treatments could be explained by prior vegetation removal (1), differences in SOM (2), current vegetation cover (3), and soil water content (4):

(1) Earthwork (removal of vegetation) removed all *C. ladanifer* from the field, which may explain the higher number of individuals in the Control group. There was indeed a lag time (4 months) between the time the vegetation was burned and the time the field work was undertake on the plots (except for Control). However, since Machine plots host the same amount of *C. ladanifer* as the Control (Figure 12), the creation of swales, which are more destructive to the soil, seems to have affected the abundance the most. *C. ladanifer* is indeed an obligate seeder (Silva Dias et al., 2019) and the passage of rippers can reverse soil layers (de Figueiredo et al., 2012), leading to a lower proportion of *C. ladanifer*'s seeds in the topsoil.

(2) Since SOM accelerates plant establishment, this may explain why more *C .ladanifer* grew in the Control and Machine plots (Martínez et al., 2003). However, the presence of *C. ladanifer* may also explain the high SOM content in Machine, as shrubs can contribute to additional carbon sequestration and thus increase soil carbon stocks (Frazão et al., 2018; Ruiz-Peinado et al., 2013). In Mediterranean areas, the contribution of shrub layer to the carbon stock ranges from 29 to 20% (Ruiz-Peinado et al., 2013).

(3) Control, Machine, and Swales had lower soil moisture content than the other treatments in December 2021. Since *C. ladanifer* is a species that can develop in poor and bare soils, this could explain the high number of this species in those plots. Their presence can displace other species that cannot develop in such extreme conditions (Larchevêque et al., 2005). The effect may also be the catalytic, since a dense shrub layer can lower the water content of the soil (Bohlman et al., 2016). On the other hand, other studies (Castro et al., 2002; Ramos Solano et al., 2007b) state that shrubs promote surrounding vegetation by providing shade, soil moisture and nutrients, and improving soil properties. But since water content and abundance of *C. ladanifer* were also lower in the plots treated with swales only (Figure 12), this second hypothesis seems more probable.

(4) Because GVC is higher in the Control plots (Figure 8) and *C. ladanifer* is a common species throughout the vegetation, there is also a higher likelihood that more *C. ladanifer* will be found in these plots. However, the GVC in the Machine is statistically lower than in the Control, but the abundance of *C. ladanifer* is the same, even slightly higher (Figure 12). So, the proportion of *C. ladanifer* is more important in Machine than in Control. Since SOM is also lower in Machine than in Control, this suggests that native vegetation overall increases SOM, but not *C. ladanifer* itself (which was confirmed since there was no correlation between SOM and *C. ladanifer* abundance).

C. ladanifer also gives an indication of the influence of treatment on plant nutrition, since plant phenological characteristics are generally better in plots treated with organic amendment, as shown in Figure 17 (Compost and Woodchips). The parameters most affected were leaf area (Cistus LA), plant height (Cistus H), and plant moisture (Cistus M). Organic amendment and higher soil water content (which was higher in the plots Woodchips and Compost in December 2021, see Appendix 7) improve the ability of plants to retain water and thus grow (Larchevêque et al., 2006; Mugnai et al., 2007). Compost and woodchips can increase plant growth thanks to

the nutrient they bring (Larcheveque et al., 2006) and their ability to reduce runoff and loss of nutrients, which also improves the environment for plant growth (Prats et al., 2014). Moreover, there is less native vegetation in general in plots where *C. ladanifer* parameters are higher, thus less competitivity and easier access to water and nutrient for plants (Figure 8 and Figure 12).

4.3. Tree and shrubs survival rates

Seedlings survival in the compost-treated plots was significantly higher than in Machine and Swales (Figure 14). There is also a tendency in Woodchips-treated plots to have higher survival rates, although this could not be demonstrated statistically. So, the seedlings with higher survival rates were planted in the swales (OS) where a thick layer of organic material was applied. Thus, the layer of organic matter was the factor that most helped the trees survive. It can indeed bring nutrients to the soil (Larcheveque et al., 2006; S. Prats et al., 2014), simulating the uptake of macro- and micronutrients by plants (Guerrero et al., 2001), increase soil infiltration rates (Díaz et al., 2022), and thus increasing their survival rate.

However, SOM did not differ between the treatments for the soil collected in the swales (OS), where seedlings were planted (Figure 7). Nutrient levels could nonetheless have been improved by woodchips and compost amendment, such as potassium or nitrogen (Larchevêque et al., 2006). The organic amendments could also have prevented erosion and thus cause a higher survival rate thanks to lower nutrient losses, which promotes seedling growth (Prats et al., 2014). It also helps keep the soil moister, which was confirmed in this experiment as the soil water content was higher in the Compost and Woodchip plots (Appendix 7), as well as survival rates (Figure 14). As mentioned earlier, the layer also prevented native vegetation from growing, therefore preventing potential competition with seedlings for soil water, nutrients, and light. Indeed, Bohlman et al. (2016) stated that tree seedlings require early control of competing vegetation (primarily shrubs) to achieve higher survival rates.

The highly negative and significant correlation between GVC and survival rates suggests indeed that the presence of native vegetation reduces seedlings survival rates (Figure 16). Abundance of *C. ladanifer*, which is also negatively correlated with seedlings survival, suggests the same (Figure 17). Moreover, seedling survival was low in the Machine where vegetation and *C. ladanifer* were abundant, and high in the compost where vegetation and *C. ladanifer* were not abundant. *C. ladanifer* tends to act as competing species for other plants. Indeed, they can initiate competition for water and nutrients and inhibit the growth of several other plants by releasing allelochemicals (Bohlman et al., 2016; Frazão et al., 2018). *C. ladanifer* would thus not act like a "nurse plant" for seedlings like it was hypothesized in the introduction of this work (Castro et al., 2002; Ramos Solano et al., 2007b; Ruiz-Peinado et al., 2013). However, the seedling survival samples were taken in December 2021 and the *C. ladanifer* and native vegetation samples were taken in April 2022, which may reduce the correlation coefficients and significance.

One aspect to consider is also that the probability of collecting *C. ladanifer* or to find dense cover of native vegetation near seedlings is probably very low. The vegetation is indeed sparse near the seedlings and *C. ladanifer* abundance is low in most of the planted plots. It is likely that seedlings, native vegetation and *C. ladanifer* were all affected by treatments but that there were no direct interactions between them, except in Machine plots where vegetation cover is high even where seedlings are planted (Figure 8). For example, and as it was highlighted in the

previous points, contour bunds applied in the field disfavored native vegetation cover and *C. ladanifer* abundance but did favor survival rates of seedlings.

When C. *ladanifer* is wetter, stands taller, and forms larger leaves, seedlings survival rates are also higher (Figure 12). Thus, *C. ladanifer* <u>could</u> provide an indication of whether or not the seedlings are wetter, taller, and with wider leaves. Also, the abundance of *C. ladanifer* is not affected by richer treatments, but their moisture, leaf area and height are, which indicates the positive effect of treatments. Those parameters are correlated with seedlings survival (Figure 17), which are therefore likely to have higher height and wider leaves in Compost and Woodchips plots. However, as 8 different shrub and tree were planted, they most likely conduct themselves differently under treatments.

Plant moisture's increase makes the ecosystem more resilient to wildfire, as higher moisture content in foliage leads to higher fire resistance (Agee et al., 2002). On the other hand, it also increases plant sensitivity to drought because fertilization increases the size of transpiration organs and the development of superficial roots, which are more sensitive to drought (Larchevêque et al., 2005).

5. CONCLUSION AND PERSPECTIVES

4.1. Main results

This showed that there are clear influences of soil treatments on native vegetation presence. Treatments that consisted of removing previous vegetation (Machine) resulted in a significant decrease in biomass and vegetation cover, even 1.5 years after the machine passed the field. The installation of contour bunds resulted in a lower amount of vegetation within the swales than on the ridges, where the vegetation cover and biomass were equivalent to the vegetation in the plots without contour bunds (Machine). The layer of organic material (compost or woodchips) applied within the swales seems to be the main reason for the differences in vegetation (mulching effect).

SOM is higher where no treatment was applied, i.e. Control. The addition of organic material amendment (compost or woodchips) did not show an increase in organic matter content in soils. This is mainly explained by field preparation that have probably removed burned organic materials from the soil (when removing vegetation) and reversed soil layers (when creating contour bunds). The vegetation cover, also affected by treatments (see paragraph above), affects SOM as it is higher where the soil is cover with native vegetation, i.e. Control and between swales (BS).

Native vegetation is more humid where contour bunds have been created, with slightly higher values in the amended treatments. *C. ladanifer* studied as an indicator species tends to grow wetter, taller, and have larger leaves in the plots with organic material. Contour bunds favor infiltration rates and root depth and results indicate likely higher nutrient and water content in soils that were indeed more humid four months earlier (December 2021). *C. ladanifer* abundance is also highly dependent on treatments, being more abundant in plots with Machine and Control than in plots with Swales, Woodchips and Compost

Survival of shrubs and trees planted in the field is higher in Compost and Woodchips plots than in Control plots. There is a negative correlation between the survival rate of these plants and the presence of native vegetation and *C. ladanifer.* Thus, the native vegetation and *C. ladanifer* could be competing with the seedlings. However, it is more likely that the planted trees and shrubs survived better in the Compost and Woodchips areas because the organic matter amendment protected them from possible competition from other plants, but also increased soil moisture, protected the soil from erosion, and likely added nutrients to the soil that are important for plant growth.

Finally, the model construction using a linear regression between biomass and GVC showed that GVC is a good proxy to estimate the biomass. Moreover, the calculation of GVC with Canopeo demonstrates that it could be an accurate, rapid, and inexpensive tool to calculate native vegetation biomass in the field. It would be interesting to add the mean or median height of the vegetation in the field to get a more accurate estimate.

4.2. Perspectives for landowners

If the landowner's main objective is to rapidly increase biomass on his land after a forest fire (revegetation), no treatments should be applied. *C. ladanifer* would probably be abundant in such case and provide dietary supplement for ruminants. However, this work does not provide information about the diversity of vegetation and thus future biodiversity in the field, which could be affected in the long term

If the landowner's primary goal is to grow plants with higher humidity that are more resistant to future wildfires, the ditches swales should be constructed. Vegetation with a higher water content would also likely be more diverse. However, wetter vegetation is more sensitive to drought conditions and is therefore at risk during the summer. Therefore, it is important to monitor weather and vegetation when droughts are anticipated

If the landowner's primary goal is to reforest and create a diverse and rich forest ecosystem, swales and organic amendments should be used. Removal of vegetation and application of compost and woodchips will indeed allow for better survival results.

4.3. Outlooks and perspectives

This study has shown that parameters other than those tested could have influenced the characteristics of the native vegetation, *C. ladanifer* and the seedlings planted for the project. For example, only the abundance and cover of native vegetation was tested, not diversity, which could be more important for ecosystem regeneration after wildfire, depending on the objectives. Since the land is sloping, it would also be interesting to see if erosion differs between treatments and affects SOM rates and vegetation parameters. Finally, the contribution of woodchips or compost also brings nutrients to the soils that were not studied in this work but certainly have an impact on soil and vegetation.

This work showed that the construction of a tool to predict tree survival rates with *Cistus*-specific parameters would be possible, as this species responds positively to organic amendments. One could also think of thresholds for plant moisture above which we would see higher survival rates in seedlings. This would allow landowners to have an indication of good or poor reforestation conditions prior to reforestation and when *C. ladanifer* is present on their land. Finally, it would be interesting to examine species-specific survival rates of planted trees and shrubs to see which trees and shrubs would potentially behave like the *C. ladanifer.*

The model between vegetation cover and biomass could easily be improved by sampling quadrats of the same area (or cropping pictures already taken) and taking flower's color in account to increase the correlation between GVC and biomass. Adding the mean or median vegetation's height values would also certainly improve the tool. Once the model is improved, it would then be sufficient to measure the mean or median height of vegetation in the field in x points and photograph the vegetation in x points to obtain an estimation of biomass.

Finally, this work has many chapters and has examined many variables. Further investigation and statistical tests should be performed for almost all of them to reach suitable scientific findings. This work can be considered as a preliminary work of investigation on native vegetation and contour bunds effects within the R3forest project

LITERATURE

Abramoff, M. D., Magalhães, P. J., & Ram, S. J. (2004). Image processing with ImageJ. *Biophotonics International*, *11*(7), 36-.

Agee, J. K., Wright, C. S., Williamson, N., & Huff, M. H. (2002). Foliar moisture content of Pacific Northwest vegetation and its relation to wildland fire behavior. *Forest Ecology and Management*, *167*(1), 57–66. https://doi.org/10.1016/S0378-1127(01)00690-9

Albaladejo, J., Martinez-Mena, M., Roldan, A., & Castillo, V. (1998). Soil degradation and desertification induced by vegetation removal in a semiarid environment. *Soil Use and Management*, *14*(1), 1–5. https://doi.org/10.1111/j.1475-2743.1998.tb00602.x

Aranda, V., & Oyonarte, C. (2005). Effect of vegetation with different evolution degree on soil organic matter in a semi-arid environment (Cabo de Gata-Níjar Natural Park, SE Spain). *Journal of Arid Environments*, *62*(4), 631–647. https://doi.org/10.1016/j.jaridenv.2005.01.019

Axmanová, I., Tichý, L., Fajmonová, Z., Hájková, P., Hettenbergerová, E., Li, C.-F., Merunková, K., Nejezchlebová, M., Otýpková, Z., Vymazalová, M., & Zelený, D. (2012). Estimation of herbaceous biomass from species composition and cover. *Applied Vegetation Science*, *15*(4), 580–589. https://doi.org/10.1111/j.1654-109X.2012.01191.x

Beck, H. E., Zimmermann, N. E., McVicar, T. R., Vergopolan, N., Berg, A., & Wood, E. F. (2018). Present and future Köppen-Geiger climate classification maps at 1-km resolution. *Scientific Data*, *5*, 180214. https://doi.org/10.1038/sdata.2018.214

Bohlman, G. N., North, M., & Safford, H. D. (2016). Shrub removal in reforested post-fire areas increases native plant species richness. *Forest Ecology and Management*, *374*, 195–210. https://doi.org/10.1016/j.foreco.2016.05.008

Borcard, D. (2018). *Numerical Ecology with R* (2nd ed. 2018.). Springer International Publishing. https://doi.org/10.1007/978-3-319-71404-2

Brito, L. M., Reis, M., Mourão, I., & Coutinho, J. (2015). Use of Acacia Waste Compost as an Alternative Component for Horticultural Substrates. *Communications in Soil Science and Plant Analysis*, *46*(14), 1814–1826. https://doi.org/10.1080/00103624.2015.1059843

Castro, J., Zamora, R., Hódar, J. A., & Gómez, J. M. (2002). Use of Shrubs as Nurse Plants: A New Technique for Reforestation in Mediterranean Mountains. *Restoration Ecology*, *10*(2), 297–305. https://doi.org/10.1046/j.1526-100X.2002.01022.x

Chung, Y. S., Choi, S. C., Silva, R. R., Kang, J. W., Eom, J. H., & Kim, C. (2017). Case study: Estimation of sorghum biomass using digital image analysis with Canopeo. *Biomass & Bioenergy*, *105*, 207–210. https://doi.org/10.1016/j.biombioe.2017.06.027

de Figueiredo, T., Fonseca, F., & Martins, A. (2012). Soil loss and run-off in young forest stands as affected by site preparation technique: A study in NE Portugal. *European*

Journal of Forest Research, *131*(6), 1747–1760. https://doi.org/10.1007/s10342-011-0581-6

De Luís, M., Francisca García-Cano, M., Cortina, J., Raventós, J., Carlos González-Hidalgo, J., & Rafael Sánchez, J. (2001). Climatic trends, disturbances and short-term vegetation dynamics in a Mediterranean shrubland. *Forest Ecology and Management*, *147*(1), 25–37. https://doi.org/10.1016/S0378-1127(00)00438-2

Dentinho, T., Navas, D., & Potes, J. (2005). Chemical and nutritional evaluation of food complements for large cattle breeding, in Montado de Azinho area. *Pastagens e Forragens*, 41–46.

Díaz, M. G., Lucas-Borja, M. E., Gonzalez-Romero, J., Plaza-Alvarez, P. A., Navidi, M., Liu, Y.-F., Wu, G.-L., & Zema, D. A. (2022). Effects of post-fire mulching with straw and wood chips on soil hydrology in pine forests under Mediterranean conditions. *Ecological Engineering*, *182*. https://doi.org/10.1016/j.ecoleng.2022.106720

Fabrizio, A., Tambone, F., & Genevini, P. (2009). Effect of compost application rate on carbon degradation and retention in soils. *Waste Management*, *29*(1), 174–179. https://doi.org/10.1016/j.wasman.2008.02.010

FAO. (2015). *FAO/UNESCO Soil Map of the World | FAO SOILS PORTAL | Food and Agriculture Organization of the United Nations*. https://www.fao.org/soils-portal/data-hub/soil-maps-and-databases/faounesco-soil-map-of-the-world/en/

Frazão, D. F., Raimundo, J. R., Domingues, J. L., Quintela-Sabarís, C., Gonçalves, J. C., & Delgado, F. (2018). Cistus ladanifer (Cistaceae): A natural resource in Mediterranean-type ecosystems. *Planta*, *247*(2), 289–300. https://doi.org/10.1007/s00425-017-2825-2

Gomez-Aparicio, L., Zamora, R., Gomez, J. M., Hodar, J. A., Castro, J., & Baraza, E. (2004). Applying plant facilitation to forest restoration: A meta-analysis of the use of shrubs as nurse plants. *Ecological Applications*, *14*(4), 1128–1138. https://doi.org/10.1890/03-5084

Guerreiro, O., Dentinho, M. T. P., Moreira, O. C., Guerra, A. R., Ramos, P. A. B., Bessa, R. J. B., Duarte, M. F., & Jerónimo, E. (2016). Potential of Cistus ladanifer L. (rockrose) in small ruminant diets—Effect of season and plant age on chemical composition, in vitro digestibility and antioxidant activity. *Grass and Forage Science*, *71*(3), 437–447. https://doi.org/10.1111/gfs.12188

Guerrero, C., Gómez, I., Moral, R., Mataix-Solera, J., Mataix-Beneyto, J., & Hernández, T. (2001). Reclamation of a burned forest soil with municipal waste compost: Macronutrient dynamic and improved vegetation cover recovery. *Bioresource Technology*, *76*(3), 221–227. https://doi.org/10.1016/S0960-8524(00)00125-5

Heiri, O., Lotter, A. F., Lemcke, G., Palaeobotany and Palynology, & Dep Biologie. (2001). Loss on ignition as a method for estimating organic and carbonate content in sediments: Reproducibility and comparability of results. *Journal of Paleolimnology*, *25*(1), 101-. https://doi.org/10.1023/A:1008119611481

IBM. (2020). *Transforming Variable to Normality for Parametric Statistics* [CT741]. https://www.ibm.com/support/pages/transforming-variable-normality-parametric-statistics

Kruse, R., Bend, E., & Bierzychudek, P. (2004). Native plant regeneration and introduction of non-natives following post-fire rehabilitation with straw mulch and barley seeding. *Forest Ecology and Management*, *196*(2), 299–310. https://doi.org/10.1016/j.foreco.2004.03.022

Larchevêque, M., Ballini, C., Korboulewsky, N., & Montes, N. (2006). The use of compost in afforestation of Mediterranean areas: Effects on soil properties and young tree seedlings. *The Science of the Total Environment*, *369*(1–3), 220–230. https://doi.org/10.1016/j.scitotenv.2006.04.017

Larchevêque, M., Montès, N., Baldy, V., & Dupouyet, S. (2005). Vegetation dynamics after compost amendment in a Mediterranean post-fire ecosystem. *Agriculture, Ecosystems & Environment*, *110*(3), 241–248. https://doi.org/10.1016/j.agee.2005.04.006

Lati, R. N., Filin, S., & Eizenberg, H. (2011). Robust methods for measurement of leaf-cover area and biomass from image data. *Weed Science*, *59*(2), 276–284.

Martínez, F., Cuevas, G., Calvo, R., & Walter, I. (2003). Biowaste Effects on Soil and Native Plants in a Semiarid Ecosystem. *Journal of Environmental Quality*, *32*(2), 472–479. https://doi.org/10.2134/jeq2003.4720

Mateus, P., & Fernandes, P. M. (2014). Forest Fires in Portugal: Dynamics, Causes and Policies. In *Forest Context and Policies in Portugal* (pp. 97–115). Springer International Publishing. https://doi.org/10.1007/978-3-319-08455-8_4

Millot, G. (2014). *Comprendre et réaliser les tests statistiques à l'aide de R: Manuel de biostatistique* (3e édition). De Boeck.

Mugnai, S., Pasquini, T., Azzarello, E., Pandolfi, C., & Mancuso, S. (2007). Evaluation of Composted Green Waste In Ornamental Container-Grown Plants: Effects on Growth and Plant Water Relations. *Compost Science & Utilization*, *15*(4), 283–287. https://doi.org/10.1080/1065657X.2007.10702345

Mwendwa, S., Mbuvi, J., Kironchi, G., & Gachene, C. (2020). A geolpedological approach to soil classification to characterize soils of upper Kabete campus field. *University of Nairobi, Kenya*. htttps://www.semanticscholar.org/paper/The-Mwendwa-Protocol%3A-A-modification-of-the-method-Mwendwa/da2bce056fb92c764329ea9a55ba11e48dbf088f

Nunes, A. N. (2012). Regional variability and driving forces behind forest fires in Portugal an overview of the last three decades (1980–2009). *Applied Geography (Sevenoaks)*, *34*, 576–586. https://doi.org/10.1016/j.apgeog.2012.03.002

Patrignani, A., & Ochsner, T. E. (2015). Canopeo: A Powerful New Tool for Measuring Fractional Green Canopy Cover. *Agronomy Journal*, *107*(6), 2312–2320. https://doi.org/10.2134/agronj15.0150

Peña, E. A., & Slate, E. H. (2006). Global Validation of Linear Model Assumptions. *Journal of the American Statistical Association*, *101*(473), 341–354. https://doi.org/10.1198/016214505000000637

Poole, M. A., & O'Farrell, P. N. (1971). The Assumptions of the Linear Regression Model. *Transactions of the Institute of British Geographers*, *52*, 145–158. https://doi.org/10.2307/621706

Pouyat, R. V., Page-Dumroese, D. S., Patel-Weynand, T., & Geiser, L. H. (2020). *Forest and Rangeland Soils of the United States under Changing Conditions: A Comprehensive Science Synthesis*. Springer International Publishing AG.

Prats, S. A., González-Pelayo, Ó., Silva, F. C., Bokhorst, K. J., Baartman, J. E., & Keizer, J. J. (2019). Post-fire soil erosion mitigation at the scale of swales using forest logging residues at a reduced application rate. *Earth Surface Processes and Landforms*, *44*(14), 2837–2848. https://doi.org/10.1002/esp.4711

Prats, S., Malvar, M., Martins, M. A. S., & Keizer, J. J. (2014). Post-fire soil erosion mitigation: A review of the last research and techniques developed in Portugal. *Cuadernos de Investigación Geográfica*, *40*(2), 403–428. https://doi.org/10.18172/cig.2519

Rama, H.-O., Roberts, D., Tignor, M., Poloczanska, E. S., Mintenbeck, K., Alegría, A., Craig, M., Langsdorf, S., Löschke, S., Möller, V., Okem, A., Rama, B., & Ayanlade, S. (2022). *Climate Change 2022: Impacts, Adaptation and Vulnerability Working Group II Contribution to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*. https://doi.org/10.1017/9781009325844

Ramos Solano, B., Pereyra de la Iglesia, M. T., Probanza, A., Lucas García, J. A., Megías, M., & Gutierrez Mañero, F. J. (2007a). Screening for PGPR to improve growth of Cistus ladanifer seedlings for reforestation of degraded mediterranean ecosystems. In E. Velázquez & C. Rodríguez-Barrueco (Eds.), *First International Meeting on Microbial Phosphate Solubilization* (pp. 59–68). Springer Netherlands. https://doi.org/10.1007/978-1-4020-5765-6_7

Ramos Solano, B., Pereyra de la Iglesia, M. T., Probanza, A., Lucas García, J. A., Megías, M., & Gutierrez Mañero, F. J. (2007b). Screening for PGPR to improve growth of Cistus ladanifer seedlings for reforestation of degraded mediterranean ecosystems. In E. Velázquez & C. Rodríguez-Barrueco (Eds.), *First International Meeting on Microbial Phosphate Solubilization* (pp. 59–68). Springer Netherlands. https://doi.org/10.1007/978-1-4020-5765-6_7

R3forest. (2021). Using exotic biomass for post-fire recovery: Reuse, Regenerate and Reforest. [Leaflet].

RDocumentation. (2022). *pairwise.t.test function—RDocumentation*. https://www.rdocumentation.org/packages/stats/versions/3.6.2/topics/pairwise.t.test

Ruiz-Peinado, R., Moreno, G., Juarez, E., Montero, G., & Roig, S. (2013). The contribution of two common shrub species to aboveground and belowground carbon stock in Iberian

dehesas. *Journal of Arid Environments*, *91*, 22–30.
https://doi.org/10.1016/j.jaridenv.2012.11.002

Rundel, P. W., Arroyo, M. T. K., Cowling, R. M., Keeley, J. E., Lamont, B. B., Pausas, J. G.,
& Vargas, P. (2018). Fire and Plant Diversification in Mediterranean-Climate
Regions. *Frontiers in Plant Science*, *9*, 851–851.
https://doi.org/10.3389/fpls.2018.00851

Schmidt, A. F., & Finan, C. (2018). Linear regression and the normality assumption. *Journal
of Clinical Epidemiology*, *98*, 146–151. https://doi.org/10.1016/j.jclinepi.2017.12.006

Silva Dias, L., Pires Pereira, I., & Soveral Dias, A. (2019). Seed Germination in Cistus
ladanifer: Heat Shock, Physical Dormancy, Soil Temperatures and Significance to
Natural Regeneration. *Plants (Basel)*, *8*(3), 63-. https://doi.org/10.3390/plants8030063

Turco, M., Jerez, S., Augusto, S., Tarín-Carrasco, P., Ratola, N., Jiménez-Guerrero, P., &
Trigo, R. M. (2019). Climate drivers of the 2017 devastating fires in Portugal.
Scientific Reports, *9*(1), 13886–13888. https://doi.org/10.1038/s41598-019-50281-2

United Nations. (2016). *Transforming our world: The 2030 Agenda for Sustainable
Development | Department of Economic and Social Affairs*.
https://sdgs.un.org/2030agenda