GREEN ROOFS IMPLEMENTATION AND ASSESSMENT IN COASTAL AREAS

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Biologia Funcional e Biotecnologia de Plantas

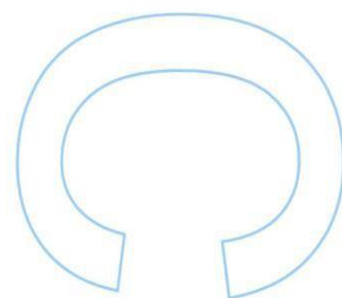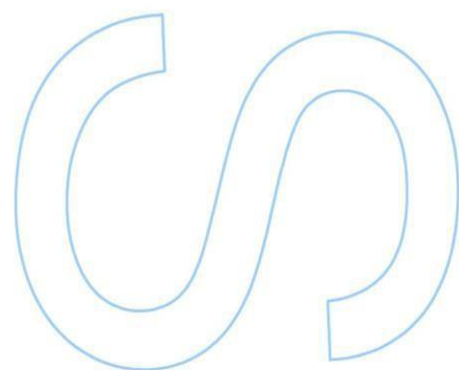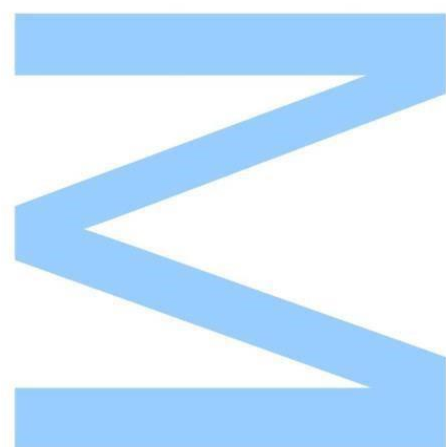Departamento de Biologia

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"Every person must work for his own improvement, and at the same time he must share a general responsibility for all humanity"

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Abstract

This thesis was developed from a multifunctional approach of green roof systems. Its main goal was to provide information about the viability of the selected components when facing coastal conditions. The main attention relies on plant establishment and consequent interactions between plants and substrate. Those in turn will affect microbial communities composition, water runoff quality and temperature variations which correspond to our subsequent topics of analysis. In the following green roof systems, an innovative drainage layer was used, insulation cork board (ICB), as an alternative to the conventional materials.

Two experiments were setup in order to go towards our main goal.

In experiment I non-vegetated (Control) and vegetated (*Ammophila arenaria, Corema album, Helichrysum italicum* and polyculture) green roof systems were designed with commercial substrate and coastal plants as mono and polyculture. The results from this experiment show that the interactions between plant and substrate influence microbial communities within the substrate and water runoff quality. Among the tested plants *A. arenaria* showed the best survival capacity. *C. album* showed low development and *H.italicum* did not prosperate. From substrate and rhizosphere samples, the dominant phylum (*Proteobacteria, Bacteroidetes*, *Actinobacteria*, *Planctomycetes*, *Acidobacteria*, *Chloroflexi*, *Verrucomicrobia*, *Opisthokonta*, *SAR* and *Gemmatimonadetes*) registered by next generation sequencing of the 16S rRNA gene targeting the V4-V5 hypervariable regions, presented low variations between systems and over time. Therefore, our results indicate that under the coastal conditions, the substrate composition was the main factor influencing microbial phylum abundance. However, considering all data, different patterns between systems and over time were observed, suggesting that microbial communities structure changed with seasonality and plant species. A detailed attention in *A. arenaria* rhizosphere allowed us to observe the presence of genera affiliated with *Clostridium, Azotobacter*, *Azospirillum* and *Rhizobium*. This data highlights the potential functional characteristics that this plant may have to substrate fertilization, through nitrogen-fixing bacteria. Besides, considering the results of water quality parameters, the results show that the selected components act as a source of phosphates. Differences on water runoff phosphates concentrations were observed between vegetated and non-vegetated systems. Understanding nutrient runoff dynamics provides insight into materials selection and aid in developing ideas to minimize the risk of nutrient leaching. Furthermore, the selected components have good thermal insulation characteristics, showing prominent results for further studies.

In experiment II, we designed two different green roofs using different varieties of substrates (commercial and experimental) and three species of plants *(Calystegia soldanella, Euphorbia paralias* and *Medicago marina*) common to both substrates. The results from this experiment show that both substrates were equally suitable to roost the selected plants. Furthermore, no significant differences in water quality runoff were observed, concluding that both acted as a source of nutrients, namely phosphates and nitrates. Considering temperature variations, data obtained evince the potential thermal insulation characteristics of the systems with commercial substrate and selected plants. Furthermore, a macrofauna characterization allowed to conclude that both experiments showed potential to function as habitat for various groups of organisms (Araneae, Formicidae, Lepidoptera, Coleoptera, Culicidae, Hemiptera, Diplopoda, Coccinellidae and Orthoptera).

Hereupon, our results provide comprehensive knowledge into green roof components and their dynamics under coastal conditions and insight for further studies.

Resumo

Esta dissertação desenvolveu-se a partir de uma abordagem multifuncional de sistemas de coberturas verdes. O principal objetivo foi de dar informação acerca da viabilidade dos componentes selecionados sob as condições da zona costeira. A principal atenção recai na capacidade de adaptação das plantas e consequentes interações entre as plantas e o substrato selecionados. Estes por sua vez, vão afetar a composição das comunidades microbianas, qualidade da água escoada e variações de temperatura, que correspondem os nossos subsequentes tópicos de análise. Nos sistemas de coberturas verdes seguintes, foi utilizada uma camada de drenagem inovadora, aglomerado de cortiça expandida (ICB), como alternativa ao material convencional.

Duas experiências foram estabelecidas de forma a atingir o nosso principal objetivo.

Na experiência I, sistemas sem vegetação (Controlo) e com vegetação (*Ammophila arenaria, Corema album, Helichrysum italicum* and *polyculture*) foram construídos com um substrato comercial e plantas costeiras como mono e policultura. Os resultados desta experiência mostram que as interações entre plantas e substrato influenciam as comunidades microbianas e a qualidade da água escoada. Entre as plantas testadas *A. Arenaria* apresentou a melhor capacidade de sobrevivência, seguida de *C. album* com pouco desenvolvimento. *H.italicum* não sobreviveu. A partir das amostras de substrato e ao nível da rizosfera, os filos dominantes (*Proteobacteria, Bacteroidetes, Actinobacteria, Planctomycetes, Acidobacteria, Chloroflexi, Verrucomicrobia, Opisthokonta, SAR* and *Gemmatimonadetes*) registados por sequenciamento de 16S rRNA apresentaram pequenas variações entre os diferentes sistemas e ao longo do tempo. Assim, os nossos resultados sugerem que sob as condições da zona costeira, a composição do substrato foi o fator principal a influenciar a abundância microbiana destes filos. No entanto, diferentes distribuições entre sistemas e ao longo do tempo foram observados, sugerindo que a estrutura das comunidades microbianas muda com sazonalidade e as plantas selecionadas. Um enfoque na *A. arenaria* permitiu-nos observar a presença de generos afiliados com *Clostridium, Azotobacter, Azospirillum* e *Rhizobium*. Estes dados realçam o potencial das características funcionais que esta planta pode ter na fertilização do substrato, através de bactérias fixadoras de azoto. Além disso, considerando os resultados dos parâmetros da análise da qualidade da água, os resultados mostram que os componentes selecionados agem como uma fonte de fosfatos. Diferenças nas concentrações de fosfatos da água escoada foram observadas entre sistemas com e sem vegetação. Compreender a dinâmica do escoamento de nutrientes oferece conhecimento para a seleção de materiais e contribui

para o desenvolvimento de novas ideias para minimizar o risco de nutrientes escoados. Além disso, os componentes selecionados apresentam características de bom isolamento térmico, mostrando resultados proeminentes para estudos futuros.

Na experiência II, dois sistemas de coberturas verdes foram construídos usando diferentes substratos (comercial e experimental) com três espécies de plantas (*Calystegia soldanella, Euphorbia paralias* and *Medicago marina*) comuns aos dois substratos. Os resultados desta experiência mostraram que ambos os substratos mostraram-se adequados para suportar as plantas selecionadas. Além disso, não foram observadas diferenças significativas na qualidade da água escoada, concluindo que ambos foram uma fonte de nutrientes. Considerando as variações de temperatura, os dados obtidos evidenciam boas características de isolamento térmico dos sistemas com substrato comercial.

Além disto, a caracterização da macrofauna permitiu concluir que ambas as experiências mostraram potencial para funcionar como habitat para vários grupos de organismos (Araneae, Formicidae, Lepidoptera, Coleoptera, Culicidae, Hemiptera, Diplopoda, Coccinellidae and Orthoptera).

Posto isto, os nossos resultados dão um conhecimento abrangente acerca de componentes para coberturas verdes e a sua dinâmica sob condições de zonas costeiras e informação para estudos futuros.

Palavras-chave: Coberturas verdes, abordagem multifuncional, condições costeiras, biodiversidade, plantas costeiras, qualidade da água, variações de temperatura, ecologia do solo, estrutura microbiana.

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List of abbreviations

µS/cm	Microsiemens per centimeter
16S rRNA	16S ribosomal ribonucleic acid
$CaCl_2$	Calcium chloride
C:N	Carbon:nitrogen ratio
COD	Chemical oxygen demand
EC	Electrical conductivity
eDNA	Environmental DNA
e.g.	Exempli gratia
ETR	Relative Electron Transport Rate
FLL	Forschungsgesellschaft Landschaftsentwicklung Landschaftsbau
Km/h	Kilometers per hour
K_2O	Potassium oxide
H_2O	Water
hPa	Hectopascal
IPMA	Instituto Portuguès do Mar e da Atmosfera
mg/L	Miligrams per litter
mg/kg	Miligrams per kilo
Mm	Millimeters
M.O.	Organic matter
N	Nitrogen
n.a.	Not applicable / not available
NH_4^+	Ammonium
NH_4Cl	Ammonium chloride
nMDS	Non-metric multidimensional scaling
NO_3^-	Nitrates
NO_2^-	Nitrites
OTU	Operational taxonomic unit
P	Pressure
P_2O_5	Phosphorous pentoxide
PO_4^{3-}	Phosphates
PSII	Photosystem II
R.H.	Relative humidity
SSU rRNA	Small subunit ribosomal ribonucleic acid
T	Temperature

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Chapter I

Introduction to green roofs framework

1 INTRODUCTION

Green roofs are a technology developed taking in consideration natural environments. They are implemented on top of buildings, being constituted by several components which must ensure its good quality structure and longevity. Between those components, the substrate and the vegetation layer[1] are the ones that will support and give life to the roof, providing several benefits associated to green areas. Herein, they can be part of the green lung in cities, providing oxygen to make up for polluted areas (Schrader and Böning, 2006).

This technology has been more and more embraced because of its vast environmental, social and economic benefits, particularly in cities, because of its capability to reduce some adverse effects of rapid urbanization (Shafique *et al.*, 2018). However, green roofs implementation and establishment still pose some challenges. Since they are found mostly on the top of buildings, they may face adverse microclimatic conditions, such as high light intensity, wind and temperature extremes (Sutton, 2015). Thus, it is important to consider proper materials to each condition to assure the green roof maximum functionalities (Bevilacqua *et al.*, 2015).

Just as gardens, green roofs can be considered a dynamic system, having different components in constant interaction between them and with the surrounding environment. Those interplays will exert influence on the rain water which goes through the whole system to the downstream end (Buffam *et al.*, 2016). Hereupon, since there is a lack of comprehension regarding multifunctional approaches, combining the knowledge between green roof factors becomes more and more important. It may provide important information regarding the occurrence of trade-offs between ecosystem functions of green roofs (Lata *et al.*, 2018). Herein, conciliating the lack of knowledge of multifunctional approaches and lack of knowledge concerning the application of green roofs in coastal areas, this study will provide important information regarding components selection and their dynamics under such conditions.

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[1] "Layers" refers to the different components of green roofs

1.1   Study objectives

An overall main goal of this thesis was to evaluate the viability of the selected green roof pilot system components under the climate coastal conditions, namely: plants, substrate and technical layers. Thus, the primary objective is to assess the plant establishment capacity in selected substrates, whose both specific properties lead to different patterns of biotic and abiotic parameters. Therefore, we evaluate how different plant-substrate selections and their interactions influence water runoff[2] quality, substrate temperature and rhizosphere microbial communities. Furthermore, was used for the first time in such conditions a water drainage and retention layer made of expanded cork agglomerate (ICB – insulation cork board) as a substitute of traditional layers made of polystyrene. This will support a strategy to better select the green roof components to face coastal conditions and possible adaptations to climate change effects.

To achieve our main goal, two different experiments were conducted, with different and specific work objectives of this thesis.

In the first experiment, a set of systems with plants as monoculture and polyculture and a control with no plants, were implemented in a commercial substrate under the climate conditions of the coastline. In those systems, the plants were the differential factors in the system proprieties. Hence, we intended to:

1.   Evaluate the survival capacity and performance of different autochthonous drought-tolerant plant species in the selected substrate;

2.   Evaluate the influence of the selected components on rainwater composition and if there were significant differences on water quality between treatments;

3.   Analyze the evolution and the existing differences in microbial communities in the substrate studied under different plant covers and over time;

4.   Analyze the influence of selected components on thermal variations.

In the second experiment, a set of two systems with plants as polyculture were implemented with two different substrates: a commercial substrate and an experimental substrate. In those systems, the substrates were the differential factors in the system proprieties. Herein, we intended to:

1.   Evaluate the survival capacity and performance of the same mix of autochthonous drought-tolerant plant species under the selected substrates;

2.   Evaluate the influence of the selected components on rainwater composition and if there were significant differences on water quality between treatments;

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[2] Water drained through the green roof is referred to as runoff

3.   Analyze the influence of selected components on thermal variations.

A characterization of the climate and microclimate conditions was also carried out and a monitoring of macrofauna through *pitfall traps* was done to evaluate the potential of the systems to attract biodiversity.

1.2   Structure

This thesis is divided in three chapters. The first chapter corresponds to the introduction to the work, approaching the green roofs framework, namely its definition as a nature-based solution, importance, incentives, benefits, types, design and its impact and functioning as a system. The second part corresponds to the section of materials and methods, where are described the methodologies and materials used to accomplish each work objective. The third chapter corresponds to the results description and its respective discussion. Lastly, the chapter four corresponds to the overall conclusion and future work perspectives.

1.3 GREEN ROOFS CONCEPT

Green roofs, vegetated roofs (Hoffman and McDonough, 2005) or living roofs consist on a multilayered system with vegetation on the top, that can be placed atop various kinds of infrastructures such as buildings and parking lots (Francis and Lorimer, 2011). They are considered nature-based solutions, which according to the European Commission, are "actions that are inspired, copied or supported by nature" (EU, 2015). They employ various processes and characteristics of natural systems like the ability to balance water flow, provide oxygen and store carbon. The intention through their use is to achieve desired repercussions such as: reduction of disaster risks, build resilience, improve population well-being and embrace socially a green growth (EU, 2015).

1.3.1 Green roofs vs Conventional roofs

Globally, it is observed an increasing trend of urban population, residing in 2018, 55% of the world's population in urban areas (United Nations, 2018). This fast development of urban environments is often followed by the degradation of the environmental quality i.e. through increase in pollution (by excessive energy consumption, noise, greenhouse gases and other pollutants), development of urban heat islands (UHIs)[3], reduction of biodiversity, increase of impermeable areas and, loss of arable land and green areas (Guilland *et al.*, 2018). Hereupon, as the world continues to urbanize, the successful management of urban growth must encompass a sustainable development (United Nations, 2018).

 In 1987 the United Nations Brundtland Commission defined a sustainable development as a way of "meeting the needs of the present without compromising the ability of future generations to meet their own needs". To achieve this, policies are now supporting the 'return of nature' in cities (Guilland *et al.*, 2018), promoting preservation, restauration and/or creation of green areas, such as nature-based solutions. The intention is to minimize environmental degradation and offer different benefits that will improve the life of populations around the world. Herein, green roofs appear as an important strategy, offering a chance to provide relevant climatic, technical, ecological, social and economic advantages that conventional flat roofs cannot provide (Schrader and Bo, 2006).

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[3] Urban heat islands consist of urban areas with a higher temperature than its surrounding rural areas due to anthropogenic activities (EPA, n.a.).

1.3.2 Green roofs around the world

Green roofs technologies have a long history (e.g. Hanging Gardens of Babylon constructed around 500 BC). However, since the early 1960s, they are becoming extremely popular in several countries around the globe (Shafique *et al.*, 2018). Many countries are promoting rules and strategies for the application of green roofs on buildings, nevertheless in Portugal there are no guidelines nor reference in the legislation concerning green roofs (Calheiros and Palha, 2017). For example, in France, since 2015 it is mandatory that all commercial establishments, that are recently constructed, have a portion of the roof installed with photovoltaic panels or vegetative roofs (Vijayaraghavan *et al.*, 2019). Similarly, in Toronto, Canada, buildings with a minimum gross floor area of 2000m² must apply green roofs on 20–60% of the total roof area (Chen C-F., 2013). However, the benefits and value of green roofs are yet not recognized by several countries and their own policy makers. Lack of knowledge, initial high construction costs, addition of extra weight to buildings, maintenance and roof leakage problems are between the main obstacles associated with the application of green roofs (Shafique *et al.*, 2018; Sutton, 2015).

In order to become more advantageous and cost effective than the traditional approaches, practical guidelines were created to support planning, construction needs and maintenance of green roofs. For example, Forschungsgesellschaft Landschaftsentwicklung Landschaftsbau (FLL) German guidelines are technical guides that function as a model for various studies of green roofs. They offer valuable information about the green roof technology such as how to plan and execute it. However, guidelines specific to certain climate conditions have also been developed to adapt the construction and maintenance needs to those regions (e.g. Design Guidelines and Maintenance Manual for Green Roofs in the Semi-Arid and Arid West (Tolderlund, 2010). Further research on green roofs considering local conditions will therefore support green roofs planning on its maximum efficiency (Vijayaraghavan *et al.*, 2019).

1.3.3 Benefits of green roof construction

The trust deposited by various countries on this ecotechnology, is based on various evidences showing that green roofs can provide multiple environmental, social and economic benefits, also called 'ecosystem services' [4]. Regarding those, the implementation of green roofs has the potential to provide: (1) improvement of air quality

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[4] Ecosystem services are the direct and indirect contributions of ecosystems to human well-being (BISE, n.a.).

through filtration of atmospheric pollutants by plants and surface (Currie and Bass, 2008); (2) sound insulation, acting as a barrier to sound (Renterghem and Botteldooren, 2009); (3) life extension of roof membranes through protection of ultraviolet radiation, temperatures extremes and mechanical damage (Miller, 2012); (4) precipitation filtering and stormwater runoff reductions through water retention by green roof constituents (Mentens *et al.*, 2006); (5) mitigation of urban heat and solar radiation e.g. by shading (Kosareo and Ries, 2007); (6) thermal buffering, reducing the heat flux in buildings both in summer and winter. Energy savings can be associated to the improvement of building insulation which is highly influenced by substrate and plants that provide shading, transpiration, and wind shielding. (Tabares-Velasco and Srebric, 2012; Eksi *et al.*, 2017). Furthermore, green roofs (7) may function as stepping-stones habitat[5] (Ksiazek-Mikenas *et al.,* 2018). This permits the connection between isolated habitats (e.g. park areas, gardens and graveyards) and promotes conservation of urban flora and fauna biodiversity. They may provide refugia, e.g. for insect and bird moving in cities, presenting as a potential valuable element to biological conductivity and ecological networking inside urban areas (Fig.1) (Williams *et al.*, 2014; Joimel *et al.*, 2018). However, the role of green roofs in urban wildlife bond remains questionable, being highly dependent on the surrounding areas and the specific characteristic of the building itself (e.g. height and area) (Mayrand and Clergeau, 2018).

Potential economic and social benefits associated to green spaces involve the (8) creation of employment to construction and maintenance, (9) increase of aesthetic value and (10) the creation of recreational spaces to people, encouraging socialization between building tenants, community gardens and local food production (EU, 2015; Shafique *et al.*, 2018). Besides, green roof technology has the advantage that can be used not only as a management practice for new development, but also a practicable solution for implementation in existing buildings (Shafique *et al.*, 2018).

Figure 1. Hypothesis of ecological networking inside urban areas promoted by green spaces (Mayrand and Clergeau, 2018).

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[5] Stepping stone habitats consist on a succession of disconnected habitat segments (Saura *et al.*, 2014)

1.4 GREEN ROOFS CLASSIFICATION

Green roofs, synthetically described as rooftops covered with substrate and vegetation (Catalano *et al.*, 2018) are broadly classified into extensive, semi-intensive or intensive. In figure 2 is represented the principal distinctive characteristics between them. The main distinctive elements are substrate depth that will dictate the dimension and type of vegetation and consequent needs of maintenance and irrigation (Calheiros and Palha, 2017).
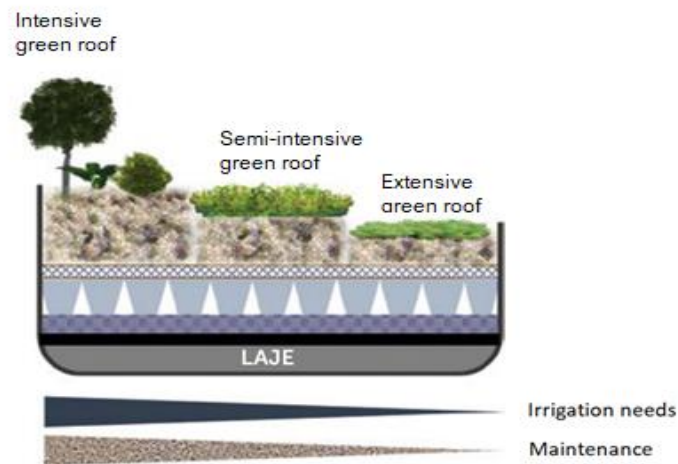
Figure 2. Schematic representation of the general structure of a green roof and classification based on the type of coverage and depth, irrigation needs and maintenance (from the left to the right: intensive, semi-intensive and extensive) (adapted from: Calheiros and Palha, 2017).

1.4.1 Intensive green roofs

Intensive green roofs are characterized by a thick substrate layer (normally around 15 - 200 cm) (Catalano *et al.*, 2018). They support a wide variety of plants including trees, shrubs, herbs and grass and therefore create an environment with additional opportunities to hold up a complex and varied ecosystem biodiversity (Catalano *et al.*, 2018; Vijayaraghavan, 2016). Because of the substrate weight and depth, they can have a similar recreation function to that of gardens and parks, being allowed public access to the installations (Calheiros and Palha, 2017; Vijayaraghavan, 2016). Comparing to gardens, the roofs will require similar (high) maintenance needs in respect of pruning, fertilization, irrigation and weeding (Catalano *et al.*, 2018).

1.4.2 Semi-intensive green roofs

In simple or semi-intensive green roofs, the plant species selection and the structural design are similar to those of intensive green roofs. Nevertheless, the execution efforts, maintenance, implementation costs and total weight exercised on the bearing structure are minor (Catalano *et al.*, 2018). Because of its reasonably thick substrate layer (12 - 100 cm) (Catalano *et al.*, 2018) they can harbor small herbaceous vegetation, small shrubs, grasses and ground covers (Calheiros and Palha, 2017; Vijayaraghavan, 2016). Regarding public access they can have moderate use (Calheiros and Palha, 2017).

1.4.3 Extensive green roofs

Extensive green roof systems are not accessible to public and are characterized by minimal maintenance and irrigation requirements. Due to the thin substrate layer (inferior to 15 cm) the water availability, nutrients and root development will be limited (Calheiros and Palha, 2017; Vijayaraghavan, 2016). Because of this, these roofs accommodate a restricted type of plant species including succulent, grasses and herbaceous, that should be capable of self-propagation (Catalano *et al.*, 2018; Vijayaraghavan, 2016). The lightweight characterized by this kind of green roofs result in a lesser structural load on the roof where they are implemented (Vijayaraghavan, 2016).

Due to building weight restrictions, maintenance and costs among the three types of green roof, the extensive type is most commonly used around the world (Vijayaraghavan, 2016). Nevertheless, green roofs in all categories can evolve into biodiverse roofs by supplying a myriad of habitats for animals and plants (Ksiazek-Mikenas *et al.,* 2018).

1.5 GREEN ROOFS DESIGN AND COMPONENTS

To be considered environmentally-friendly, to achieve optimal results and meet long-term client expectations, the selection of green roof components according to the general climate and the specific microclimatic conditions of the roof must be taken into account (Vijayaraghavan and Raja, 2014). This engineered ecosystem is frequently installed on rooftops (Fig. 3.1), where it may face extreme environmental conditions like intense solar radiation due to elevation, shading or light reflection from surrounding buildings, drought, wide temperature fluctuations and high wind speeds varying with building height and form (Papafotiou *et al.*, 2013; Oberndorfer *et al.*, 2007). Hereupon, proper design and green roof monitoring are essential for green roof projects to continue to evolve in such extreme climate conditions (Skabelund *et al.*, 2015).

However, besides climate, microclimate conditions and geographic location, component selection may vary depending on ease of sourcing of materials, cost, building type, construction detail (e.g. roof slope or orientation), life expectancy, nutrient-retention capacity, and environmental sustainability (Jennett and Zheng, 2018; FLL, 2008). Taking this in consideration, the construction of a green roof makes use of several functional layers (Fig. 3.2), combined in a way to accomplish full functionality (FLL, 2008).

(3.1)

(3.2)
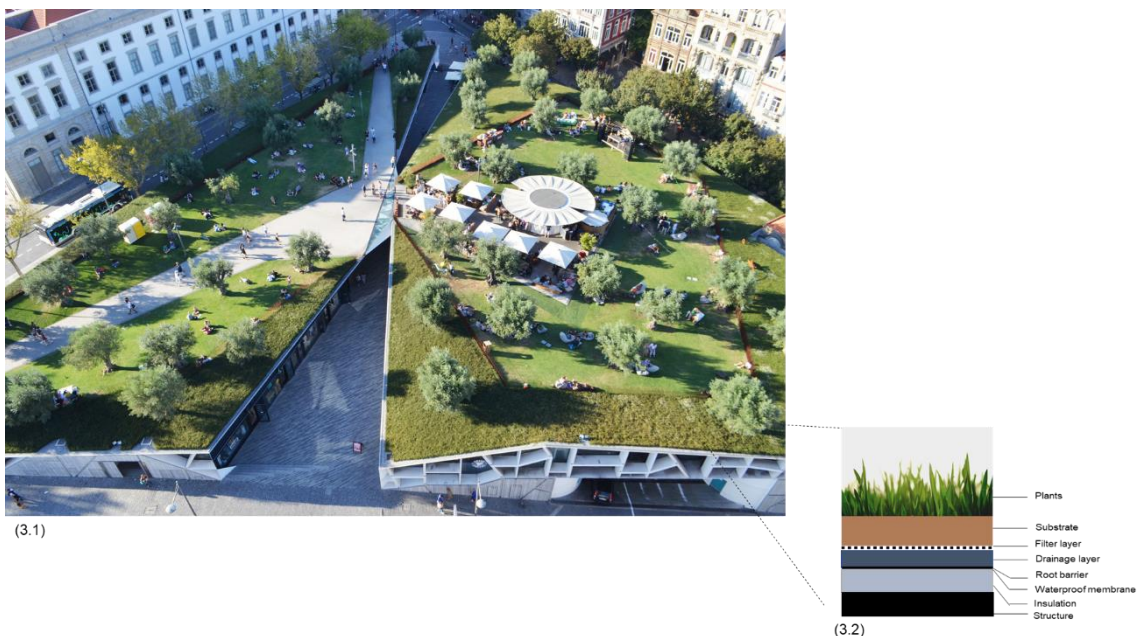
Figure 3. Example of a green roof (Praça de Lisboa, Porto) (3.1) (Photo of the author) and typical schematic representation of the green roof layers (3.2).

1.5.1 Bottom layers

In principle, a typical green roof cross-section begins at the bottom with the building's structural system and an insulation material. They are followed by a waterproofing layer, (which can be liquid-applied membranes, modified-bitumen sheets, single-ply sheet membranes and thermoplastic membranes), and a root barrier (made of metal sheets or hard plastic sheets) (Townshend, 2007). The water-proofing layer and the root barrier can be presented as one or separate components (Pérez and Coma, 2018). These components are used to protect the building from the chemical and physical influence of water and plant roots respectively, avoiding the leakage of water on the roofs and its damage by roots that otherwise could pierce from the green roof's upper layers (Perez, 2018; FLL, 2008). Above those constituents it is implemented a drainage layer, filter layer, substrate, and finally a living layer of vegetation.

1.5.2 Drainage layer

The drainage layer retains and allows the passage of water that was not retained by the vegetation, the substrate and filter layer (Perez and Coma, 2018). It also protects the underneath layers and improve thermal proprieties of green roof (Vijayaraghavan, 2016). Although is being developed new and more sustainable types of drainage layers, the two types more commonly used are:

(a)   Drainage modular panels (Fig. 4a), fabricated of plastic materials with high strength (polystyrene or polyethylene) with a cavity that allows the storage and evacuation of excess water (Perez and Coma, 2018; Vijayaraghavan, 2016);

(b)   Drainage granular materials (Fig. 4b) e.g. light expanded clay aggregates, coarse gravel and crushed bricks, have large pore spaces that provide different water holding capacities (WHC) (Perez and Coma, 2018). These materials can only be placed in slightly angled surfaces (< 5 °) or flat roofs (Vijayaraghavan, 2016);

(a)   (b)

Figure 4. (a) Example of a drainage modular panel (source: Zinco, n.a.); (b) A granular material: light expanded clay aggregates (source: MIKE WYE, n.a)

Independently of the type used, they all should provide structure and stability to the system, providing an equilibrium between water and air in the green roof system and sustain the above weight (Vijayaraghavan, 2016; FLL, 2008).

1.5.3 Filter layer

The filter layer, with high tensile strength and water permeability, is implemented between the substrate and the drainage layer. Besides functioning as a root barrier it allows the passage of water to the subsequent layers (Vijayaraghavan, 2016). However, it prevents the clogging of the drainage layer by restricting the entry of smaller particles such as plant debris and soil fine (Shafique, 2018). The materials used more commonly are polyester geotextile felts or polypropylene that should be resistant to weathering, mechanical stress, microorganisms degradation and chemicals (Perez and Coma, 2018; FLL, 2008).

1.5.4 Substrate layer

The use of pure soil or use of locally available garden/potting soil and compost was a frequent practice in the past, and where commercial substrates were not available. However, it was observed that an artificial substrate with a proper design and specifications contradict some of the negatives aspects of using 100% compost such as problems of low water retention and aeration, fast nutrient leaching, growth of unnecessary weeds, compaction and weight (Xiao *et al.*, 2014).

Many ecosystem services provided by green roofs are directly correlated with the chemical and physical properties of growth substrate. Herein, the capacity of retaining water, permeability, density, granulometric particle distribution, porosity, nutrient holding capacity, pH, electrical conductivity (EC) are some of the substrate components characteristics which affect the whole system, including water quality, thermal insulation, plant survival and microorganisms establishment (Skabelund *et al.,* 2015; Vijayaraghavan, 2016). Therefore, the optimum selection of this growth medium becomes crucial for the success of any green roof (Vijayaraghavan, 2016)

Due to climatic differences and plant species selected, there is no optimal substrate to green roofs in all regions (Ampim *et al.,* 2010); however, as proposed by the German guideline FLL (2008), green roofs substrates should combine the following properties: high stability to resist decomposition and erosion caused by rain water, wind or frost; have on its constitution components that can retain water and keep it available to plants; good oxygen diffusion; appropriate pH; low salt content; provide nutrients and physical

support to plants and be as light weight as possible to not exceed the load bearing capacity of the roof. In order to achieve all these requirements, multiple compositions of specific substrates for green roofs have been accomplished. The usual practice is to mix different components that offer the essential properties to the plant growth. They are commonly separated into organic fraction and inorganic fraction (Pérez and Coma, 2018). In the inorganic fraction, numerous types of materials can be used, such as ash, zeolite, shale, perlite, expanded clay (Fig. 5a), volcanic rock (such as scoria, pumice or pozzolan), vermiculite, sand (Fig. 5b), and even recycled materials such as porcelain, crushed bricks, and tiles (Ampim *et al.*, 2010; Pérez and Coma, 2018). All these materials are used by its porosity, permeability, compaction avoidance and lightness although to different extents (Ondoño, 2015a). Regarding to the organic fraction is usual the use of peat (Fig.5c) or compost from recycled organic waste e.g. pine bark (Fig. 5d) and coir, that can be generated from different anthropic activities (e.g. agricultural, forestry, or industrial) (Ondoño, 2015a; Pérez and Coma, 2018). This fraction serves as fuel to plants and microorganisms aiming to promote soil biodiversity and continuous cycling of nutrients. Besides, having direct impact on thermal conductivity and water retention capacity, it influences the growth conditions, water availability and nutrients necessary for plant development (Best *et al.*, 2015). The final selection will depend on the material availability, building capacity, selected vegetation, and price (Roth-Kleyer, 2001).

Hence, as substrate are one of the most important components in the construction of green roofs (Noya *et al.*, 2017), it is necessary a continuous study regarding the influence that components of the substrate can have on the green roof system and services.

Figure 5. Examples of components present on artificial substrates. (a) Light expanded clay 2-4 mm (photo of the author); (b) Sand (photo of the author); (c) Pine bark humus 0-15mm (source:Bruning group, n.a.); (d) Blonde peat (source: Lambert Peat Moss, n.a.).

1.5.5 Vegetation layer

Plants contribute to the majority of benefits of green roofs, involving: increase of aesthetic value to the building, improvement of air quality, substrate cooling by shading, decrease in urban heat-island effect, storm water peak attenuation, protect and hold the substrate from erosion (Oberndorfer *et al.*, 2007), limit weed abundance (Levine *et al.*, 2004), can promote biological N fixation, retention of soil nutrients and C, change substrate composition (Lundholm and Williams, 2015), can improve water runoff (Vijayaraghavan *et al.*, 2018), and may contribute to the quality of ecosystems through species preservation and by potentiating biodiversity (Caneva *et al.*, 2015). Although plants contribute to very important green roofs ecological services, plant community survival

highly depends on the substrate selected, climate and the microbial community development (Skabelund *et al.,* 2015).

Regarding to German guideline FLL (2008), some important plant characteristics for the extensive type of green roofs include: resistance to airborne chemical contamination, drought resistant in both cold, dry winters and hot, dry summers, wind resistant, thriving through many years (perennial plants[6]), capable of self-propagation, shallow spreading roots to avoid damages to the system, low nutritional requirements, good ground coverage, low maintenance, adapted to shallow substrates, light weight at maturity, non-invasive species, compact, and low dry matter content to moderate fire safety concerns. Hereupon, selecting adequate plant species becomes a central topic to consider in green roof design since they need to be well suited to subsist in the full range of conditions that they are expected to face.

Succulent plants, such as *Sedum* genera (Family: Crassulaceae), are between the most used worldwide in green roofs (Pérez and Coma, 2018). Their wide use and acceptance in green roofs it's a result of their unique adaptations to stress. This is as a result of particular characteristics e.g. shallow root systems, water accumulation in leaves or stems and their crassulacean acid metabolism (CAM) photosynthetic pathway (Benvenuti and Bacci, 2010). Through this last adaptation, a drought adaptation mechanism, plants take advantage of the lower temperature and frequent higher humidity during the night. They open the stomas and store CO_2, reducing water loss and increasing water use efficiency (Fang and Xiong, 2015; Ting, 1985). However, selecting different life forms, avoiding monocultures simplicity, has been showing potential to provide more efficient ecosystem functioning, mostly owing to niche complementarity or facilitation (Lundholm *et al.*, 2010).

1.5.5.1 Native plants in green roofs

Native or autochthonous plants comprise species which are natural from the region where they habit, they develop and propagate naturally. Since they are adapted to local conditions, creating green roofs with diverse and local plant species whenever possible may lead not only to pests and diseases resistance, less irrigation, fertilization and maintenance but may also enhance pollination, food and habitat resources for native insects and birds, potentiating biodiversity conservation (MacIvor and Lundholm, 2011; Brenneisen, 2006; McKinney, 2002). However, since the growing conditions on green

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[6] Perennial plants have life cycle longer than two years

roofs are different from those on the ground, the use of native plants it's not always suitable for green roofs (Oberndorfer *et al.*, 2007). The lack of availability and experience at nurseries and the difficulty in seeds germination on rooftops can also be mentioned as limitations to the use of various native plants (MacIvor and Lundholm, 2011; White and Snodgrass, 2003).

1.5.5.2 Coastal plants

Plants established in coastal environments may be considered appropriate for green roofs because of their adaptation to a large range of environmental stresses such as: (1) sandy soils with low water-holding capacity, which can lead to (2) moisture deficiency, (3) salinity, (4) extreme events such storms, (5) strong winds, (6) exposure to high light intensity, (7) high temperatures and (8) low nutrient content. The common responses and various morphological and physiological adaptations that help them to overcome such conditions include: osmotic regulation, root adaptions, change in evaporation rates, roll of leaves and hairs occurrence, phototropism, the C4 and CAM photosynthetic pathways, development of a epicuticular wax layer, succulence, salt bladders, nitrogen fixation via rhizosphere bacterial activity, phosphorus uptake via endomycorrhizae fungi, variation of life cycle and germination strategies (Hesp, 1991; Nagase and Tashiro-ishii, 2018; Nellis, 1994).

Furthermore, as coastal plants become endangered by abnormal processes, occurring significant interrelationships between direct (e.g. habitat fragmentation) and indirect anthropogenic pressure (e.g. the impact on climate change influence sea-level rise[7] and temperature changes that will affect respectively the habitat and reproduction cycle of plants), conservation efforts became crucial in many countries worldwide. Hereupon, green roofs using regional and dune plants can be part of nature restoration as an ecosystem management tool. They could help to counteract some of the negative consequences resulted from the destruction of natural habitats, supporting legislation and policy actions to protect biodiversity of this areas of big importance in order to promote a sustainable development. (Nagase and Tashiro-ishii, 2018; Mechelen, *et al.*, 2014).

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[7] According to the Intergovernmental Panel on Climate Change (GIEC), between the 1901-2010 period, the global mean sea level rose by approximately 20 cm

1.6 CLIMATE CONDITIONS

Green roofs are dynamic systems, which are highly influenced by environmental conditions and time. In order to get the best green roof performance, during its design, besides substrate selection and plant establishment, it should be considered with particular attention the environmental and climatic conditions of the region (Sutton, 2015).

Studies involving different climate conditions, plant and substrate selection have been done, including the ones by Noya and collaborators (2017), by Ondoño (2015b) and Monteiro and collaborators (2017b), developed in a Mediterranean climate and by Graceson and collaborators (2014) in a temperate marine climate. However, to our knowledge, there is no research directed to investigate green roofs under the climate variations and extreme climate events associated to coastal areas and it's under such conditions that our study it's developed.

The study of green roofs in each climate condition can assist in the development of climate-specific guidelines and can help decision makers and landscape professionals to design and adopt regionally suitable green roofs in several scenarios (Kazemi and Mohorko, 2017).

1.7 THE PLANT-ROOT INTERFACE: THE RHIZOSPHERE

At the rhizosphere, the plant-root interface, microorganisms and plant roots share the same environment (Foth, 1991). In here, they compete for the available growth factors and at the same time, can benefit each other. Plant roots leak organic compounds and slough off cells, serving as food for microorganisms, influencing microbial biomass, species composition and activity rates (Foth, 1991; McNear, 2013). Certain components of root exudates, characteristic of the plant genome, also have a specific influence on rhizosphere microorganisms by attracting certain species and repelling others. For example, legume roots release flavonoids that specifically attract bacteria of the genus *Rhizobium* (Geurts and Franssen, 1996). The microbes are then involved in various processes such as N fixing or decomposition of organic materials, resulting in the mineralization of nutrients for root absorption. They become involved in biogeochemical cycling of carbon, nitrogen and phosphorous in soil and their physical structure (Foth, 1991) (Fig. 6). This way, microorganisms can also contribute to changing the conditions of the rhizosphere, releasing growth factors that influence the growth of the root (Frankenberger and Poth 1987) or augment root exudation (Meharg and Killham 1995). However, just as presented by Maul and Drinkwater (2010) some species of plant can reduce microbial community richness and diversity, underlining the need to study the potential that plants may or may not have to the systems.
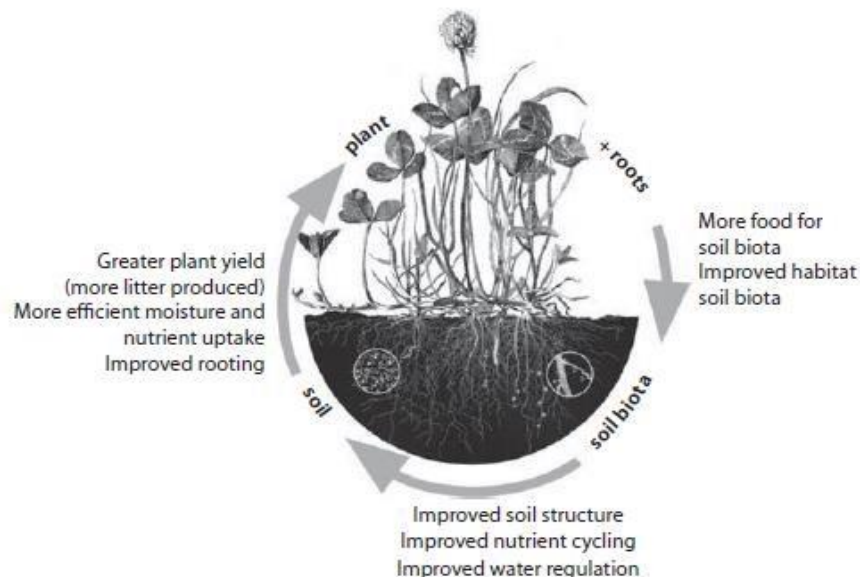
Figure 6. General cyclic interactions between plants, soil biota, and soil physical and chemical characteristics. Dead plant matter or the living plant itself can serve as food for the soil biota. in turn, the soil biota improves various processes influencing plant survival and development (Van Eekeren et al., 2007).

1.7.1 Conditions affecting plant-microbe interactions

As in terrestrial ecosystems, plant productivity depends on the nutrients present in the substrate, which in turn, its availability is heavily influenced by soil microorganisms. Bacteria and fungi are the most prevalent microbes on green roofs and the most diverse and abundant components of terrestrial soils (McGuire *et al.*, 2015). Although the plant species identity is between the main drivers of rhizosphere community composition, the structural and physicochemical environment of the rhizosphere is also influenced by substrate physical and chemical characteristics (e.g. texture, pH, degree of water saturation, nutrient and organic matter content) and environmental factors such as climate and seasonality (Marschner, 2008; Holden, 2018).

Considering this, when water supply, temperature, osmotic effects of salts, pH, nutrients and other factors are appropriate for plant growth, the conditions are generally appropriate for the development of microorganisms (Foth, 1991).

External influences such as anthropogenic influence also play a role. All these factors impact the activity and abundance of the rhizosphere community, and consequently impact plant development, growth and disease emergence (Holden, 2018). In green roofs, plants and microorganisms may face extreme abiotic conditions like aridity, strong winds, substrate thickness, high exposure to ultraviolet light and variable temperatures. These harsh conditions will affect some symbioses and by consequence, shape microbial communities and plant survival (McGuire *et al.,* 2015).

Considering this, the plant and substrate choice become key factors to green roofs since they will impact the abundance and composition of microbial communities, which may eventually affect roof function.

1.7.2 State of knowledge of microbiological analysis in green roofs

Although the importance of soil microorganisms is well-known in natural terrestrial ecosystems, there is a lack of information regarding their taxonomic diversity or functional role in green roof ecosystems (McGuire, *et al.,* 2015).

As described by McGuire and collaborators (2015) there are some functional groups of microbes that should be taken in consideration to understand green roof establishment, which comprise: mycorrhizal fungi (which develop a mutualistic relationship with plants that facilitate uptake of soil nutrients), endophytes (a diverse and protective group against plant herbivores and pathogens), decomposers (involved in nutrient cycling and degradation of organic contaminant), N-fixing bacteria (which convert atmospheric

nitrogen (N_2) to ammonia (NH_3)) and pathogens. Herein, as proposed by the study of Fulthorpe and collaborators (2018), the presence of microbial communities into green roof ecosystems has the potential to provide several benefits, such as: plant drought tolerance, protection from pathogens, access to limiting nutrients, salt tolerance, productivity and substrate stabilization. Hence, microbial characteristics become important descriptors of ecosystem quality (Ondoño *et al.*, 2014).

Although research involving the microbiome taxonomic diversity analysis in green roofs it's still scarce, Mitchell and collaborators (2018) proposed to characterize and infer about the role of microbial N cycling on green roofs through bacterial and archaeal 16S rRNA and nifH gene sequencing, concluding that plant-microbe N fixing communities could reduce the need of fertilization. Moreover, other type of studies have been accomplished. Focusing on the potential that microbes can provide to soil and plant performance, Molineux and collaborators (2016) and Young and collaborators (2015) conducted incubation experiments with soil microbes in green roofs. Their results indicate that incubation seems to be a promising method to enhancing rooftop conditions.

Most studies involving analysis of microbial community structure on green roofs focus on the analysis of the biomass (i.e. phospholipid fatty acid or PLFA analysis) and activity of the microbial community through enzymatic activities, e.g. glucosidase, phenol-oxidases, urease, and phosphatase analysis (e.g. Rumble and Gange, 2017; Molineux *et al.*, 2015; Ondoño *et al.*, 2014; Ditterich *et al.*, 2016). However, the advent of molecular microbiology tools involving the extraction and characterization of soil microbiome namely, the analysis of 16S rRNA gene sequences (a universal gene) has become frequent to assess soil microbial abundance, diversity and phylogenetic studies (Newby *et al.*, 2009). Comparing to the PLFA analysis, DNA-based methods have the advantage that can provide more information on the microbial taxa at a finer taxonomic resolution (Wurst, 2012).

Hereupon, the increasing awareness of the importance of plant–microbe interactions underlines the need to elucidate the nature of microbial communities on green roofs and the influence of biotic and abiotic factors on their establishment (McGuire, *et al.,* 2015). Consider the microbiological characteristics of the substrate, besides the common physicochemical ones, can contribute to the evaluation of substrate quality and fertility to plant development and hence increase knowledge regarding appropriate green roof components under the selected conditions (Ondoño, 2015b).

1.8 WATER RUNOFF IN CITIES

As cities expand, "green lands" disappear and impermeabilization of urban surfaces occur through, for example, the construction of roads, parking areas and rooftops. With vegetation removal and soil coverage with impermeable components there are a range of environmental functions that will be threatened. Decrease in radiation absorption, less water infiltration, more runoff, loss of biodiversity, barrier for perched water table and interrupted/reduced gas exchanges are some of the direct consequences that can lead to increased pollution, health risks, floods and subsequently higher social costs (Scalenghe and Ajmone, 2009).

1.8.1 Water cycling in extensive green roofs

On the contrary of conventional roofs, which quickly flow off rainwater, green roofs have the capacity to retain and delay the peak flow of water, reducing stormwater runoff volume (Mentens J, 2006). When precipitation falls over a green roof, the water can be held in plants, substrate and various layered materials such as drainage and water retention layers. The maximum water detention capacity will depend on the components composition and their dynamics, changing in response to atmospheric conditions such as temperature and precipitation intensity and duration (Skabelund *et al.*, 2015, Vijayaraghavan, 2016).

When the saturation point it's reached, the stage where it is not possible to retain more water in the system components, water exits the system. Transpiration and evaporation from the substrate and plant surfaces will influence the water availability in the system (Fig. 7) (Lambrinos, 2015). Hereupon, water storage in the components of the system, the flux of water between components and the exit from the system is governed by interactions between green roofs components and the physical environment (Fathi, 2017). Because of this dynamics, plant growth and water runoff quantity and quality will be influenced. With this, it is underlined the importance of the system design with adequate components considering local conditions (Lambrinos, 2015).
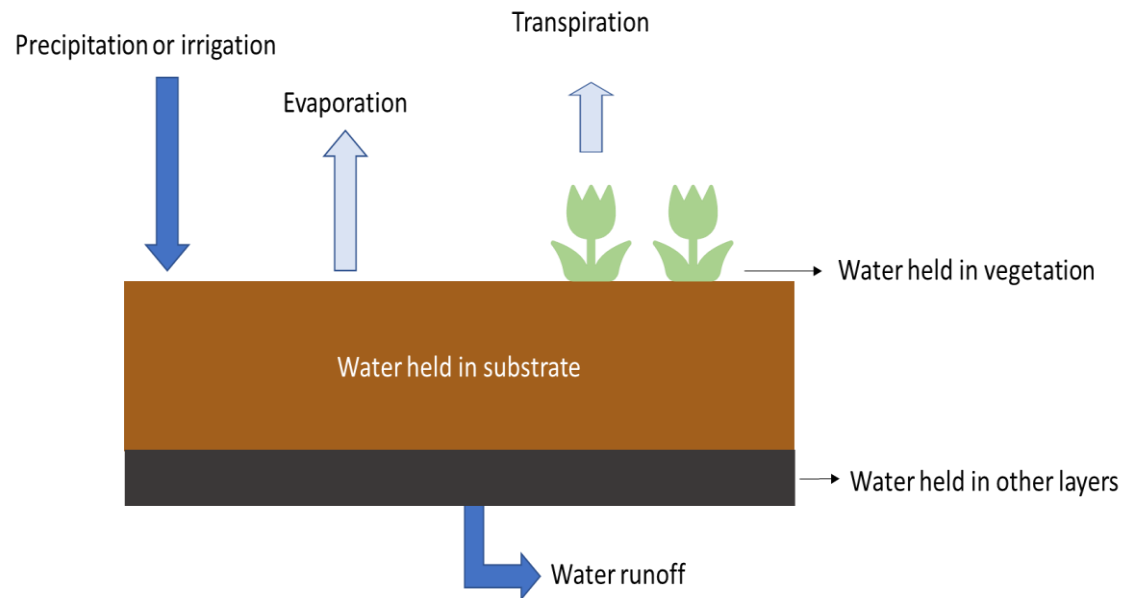
Figure 7. Water stocks and flows in green roof systems (adapted from Lambrinos, 2015).

1.8.2 Water runoff quality in green roofs

In order to green roofs function as a sustainable practice it becomes crucial to study the quality of water that percolates the system. Between the main factors influencing water runoff composition are: climate conditions, rainfall characteristics, type of pollutants from dust and airborne particulates, substrate features (e.g. depth and composition), fertilization regimes and plants selected (Vijayaraghavan, 2016). Depending on these constituents, there is a possibility for both cleaning and contaminating the water. On one side, plants and substrate can in part remove pollutants (e.g. nutrients and metals) in the rainwater by absorbing and retaining them (Vijayaraghavan *et al.*, 2019). On the other side, the percentage and type of organic matter and nutrients in substrates composition can lead to alteration in water composition (Todorov *et al.*, 2018; Vijayaraghavan *et al.*, 2012). This last case is caused by a higher concentration of charged ions in the substrate when compared to rainwater that crosses the system, consequently the runoff will have a higher concentration of those ions (Berghage *et al.*, 2009). However, water runoff chemistry, exhibits seasonal fluctuations due to changes in microbial activity, plant productivity, and other temperature or light dependent processes (Buffam *et al.*, 2016).

1.8.3 Water runoff quality analysis

There are various water quality indicators (biological, physical and chemical) that can be used to monitor and determine changes in water quality (Patil *et al.*, 2012). However, most of the studies regarding water quality runoff in green roofs focus on chemical indicators, namely, the analysis of nutrients in water runoff (e.g. Beecham and Razzaghmanesh, 2015; Vijayaraghavan *et al.*, 2012). One of the reasons is because micro- and macro-nutrients are frequently incorporated in substrates components to avoid possible limitations for plant growth (Vijayaraghavan, 2016). Once implemented, the levels of nutrients can vary with the type of green roof selected and over time owing to amounts of irrigation, precipitation event intensity and duration, roof slope and age, retention capacity, moisture, aeration, temperature, pH and other characteristics of substrate, such fertilizer regime and plant species uptake rates/productivity (Buffam and Mitchell, 2015; Harper, 2013).

1.8.3.1. Nitrogen

Nitrogen is a key macronutrient element present in amino acids, proteins, enzymes, vitamins and the nitrogenous bases of nucleic acids (Foth, 1991).

Most studies of green roofs on nitrogen had been focused on its dissolved phase, giving a lot of attention on the water runoff. As mentioned before, biotic and abiotic factors influence the nutrient content of the water runoff of green roofs. Besides (1) substrate composition and fertilizer regime having a big influence, green roof (2) vegetation may also have a strong impact in N runoff flux i.e. through plant uptake and assimilation of N forms, the reduction of runoff volume due to evapotranspiration and release of N from root exudates and litter (Fathi, 2017). Furthermore, although (3) atmospheric deposition e.g. lightning and other ionizing phenomena of the upper atmosphere may serve as a source of reactive nitrogen (4) microbially-mediated fixation of atmospheric N_2 corresponds to the main source (Foth, 1991; Galloway *et al.*, 2003; Mitchell *et al.*, 2018;). Herein, both plants and microbes can respectively assimilate and immobilize NH_4^+ and NO_3^- forms into organic pools of N, promoting the nitrogen cycling in substrates (Mitchell *et al.*, 2018).

The nitrogen cycle is comprised by four processes (Fig. 8): Fixation, ammonification and nitrification, where gaseous nitrogen is converted into usable chemical forms (ammonia or nitrate), and denitrification where fixed nitrogen is converted back to the unusable gaseous nitrogen state (Foth, 1991).

Nitrogen fixation is the conversion of molecular nitrogen (N_2) in its gaseous state to nitric oxide or ammonia. Nitric oxide can result from lightning and ultraviolet rays. However, more significant amounts of nitrogen are fixed as ammonia (NH_3) by biological fixation and subsequently into organic forms utilizable in biological processes (Encyclopædia Britannica, inc., 2019; Foth, 1991).

Ammonification, also called mineralization, is a one-way reaction in which microorganisms break down organic nitrogen and produce ammonia (NH_3). Since ammonia it is a polar gas, it can react with water, combining with a H+ and form the NH₄+ ion (Foth, 1991).

Nitrification it is a two-step process of biological oxidation of ammonium to nitrate, involving different nitrifying bacteria, being nitrite (NO_2^-) the intermediate product (Foth, 1991). On the contrary to NH_4^+ which is adsorbed onto the cation exchange sites and easily retained on the substrate, NO_3^- is more susceptible to leaching. This can lead to plant limitation of the nutrients, but also increase the risk of groundwater pollution (Foth, 1991; Mitchell *et al.*, 2018).

Denitrification is the process in which nitrate or nitrite are reduced to molecular nitrogen or nitrogen oxides by facultative anaerobes organisms. The reduced products are gaseous and frequently escape from the soil (Foth, 1991).
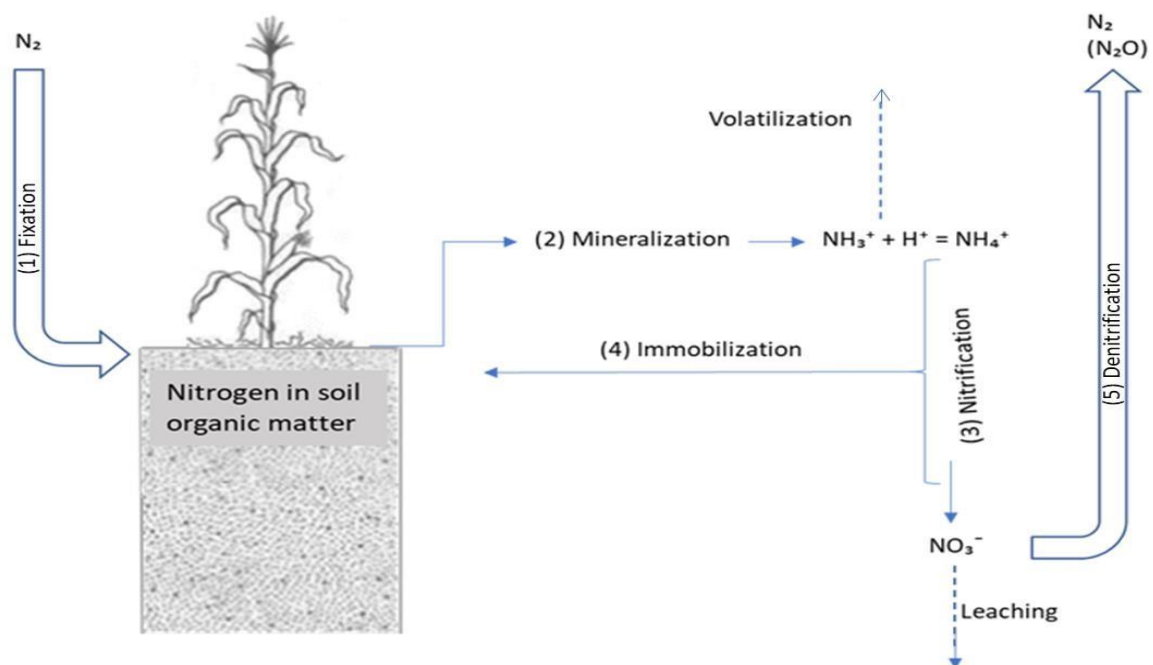
Figure 8. Representation of the major processes of the soil nitrogen cycle: Fixation, mineralization, nitrification and denitrification. Nitrogen can leave the soil by leaching and volatilization (source: Foth, 1991).

1.8.3.2 Phosphorus

Phosphorus is a key macronutrient element, being present in various constituents of cells and playing as fuel in all biochemical activities in living cells (Foth, 1991).

In urban areas, namely, in green roofs, phosphorus may come from various sources, such as: atmospheric deposition, fertilizers, soil erosion, animal wastes e.g. bird dropping, grass litter, vegetative detritus and microbial communities (Karczmarczyk *et al.*, 2017; Song, 2015; Paul, 2001; Kaye, 2006).

In natural systems, phosphorus come from the weathering of rocks and the mineralization of organic material by microbes. This allows the release of inorganic phosphate (also called orthophosphate) in a water-soluble and biologically accessible form (Foth, 1991; Mitchell *et al.*, 2017).

As with nitrogen, the phosphorus cycle involves mineralization and immobilization processes by plants and microorganisms. But on the contrary to nitrogen, the phosphate ions react rapidly with other ions in the soil solution. This results in its precipitation and adsorption in soil, leading to the conversion of phosphorus to a fixed or unavailable form. Because of this, it tends to experience slow diffusion (Foth, 1991; Mitchell *et al.*, 2017).

1.8.4 State of knowledge on water quality in green roofs

Previous studies have explored the influence of green roofs in water quality composition. For example, on his doctoral thesis, Emilsson (2005) have explored this topic, concluding that nutrients present on green roofs substrate can degrade stormwater quality. Nevertheless, have a minor influence on heavy metal runoff. Further studies have shown the same trend (e.g. (Berndtsson *et al.*, 2009; Vijayaraghavan *et al.*, 2012). However just as Harper (2013) studies report, the excess of nutrients found, may have a tendency to decline in the first few months. Still, Todorov and collaborators (2018) studies, show that due to the strong retention of water by the vegetated roof, the nutrient losses may be low, varying with seasons.

Hereupon, just as recently referred by Vijayaraghavan and collaborators (2019), it's very important to understand the potential constraint that can be associated to green roofs. Beecham and Razzaghmanesh (2015) conclude that generally the pollutant concentrations were higher in runoff from non-vegetated beds than in vegetated beds. Taking this in consideration, runoff quality could be enhanced through the right selection of green roof components.

Considering a different approach, Monteiro and collaborators (2016) results obtained from the comparison between vegetated roofs, indicate that the water that flows from the

system might be reused for non-potable purposes. Herein, just as proposed by Todorov and collaborators (2018), to overcome the problem of nutrient leaching to the surrounding environment, the water that runs out of the green roof systems could be reused e.g. for irrigation. This can contribute to an efficient water use, promoting sustainable buildings design and construction. Hereupon, this set of results and others that present the same question highlight the importance of a continuous study focused on this topic.

Chapter II

Materials and methods

2 MATERIALS AND METHODS

2.1 Site climate description

Located on the western Iberian and facing the Atlantic Ocean, according to Köppen-Geiger climate classification system, the climate of the coastal area of Matosinhos, Portugal is considered temperate (Type C). It is integrated in one of the two Cs climate varieties, classified as Csb. The Csb subtype is characterized by a temperate climate with dry and mild summers and rainy winters (IPMA, n.a.). The coastal area is characterized by daily and seasonal temperature variations, humidity, wind erosion, precipitation fluctuations and heavy storm events.

2.2 The study area and experimental set-up

The experiments were conducted in the rooftop of IPMA (Instituto Português do Mar e da Atmosfera) (Matosinhos; 41°10'49"N; 8°41'40"W), located in the first sea line (see red icon in Fig. 9). Our experimental units were placed approximately 20 meters from the beach area.

Figure 9. Local of the experiment represented as a red icon (Image obtained through google maps).

The site received shade for portions of the day due to taller buildings adjacent to the roof along the south-west to north side. During the sampling season, the roofs were exposed to direct sunlight from 6h30 to 8h30 hours per day depending on season.

Although the study was divided in two experiments, all treatments were represented by a set of triplicates of free-draining containers with the dimensions of 39x28x28 cm. Each

container was sealed with aluminum sheets to avoid extreme temperature fluctuations and a hole was created at the bottom of each container. The containers were connected through a pipe to an individual container so that the outflow water could be collected to get water samples for posterior analysis (Fig. 10). In both experiments there was no artificial watering, maintenance or removal of naturally colonizing plants.

Figure 10. Free-draining systems isolated with aluminum sheets and connected through a pipe to individual containers that retain water runoff.

Each container was built with a four-layer system comprising from the bottom to the top: an expanded cork board (ICB) (Supplier: Amorim isolamentos, S.A.) working as a water retention and drainage layer and providing thermal insulation (Fig. 11.4), a filter layer of thermoset propylene (Fig. 11.3) (Supplier: Landlab - Landl, Lda) (for more information see annex I) preventing mainly small particles from filling the drainage layer, a 12 cm substrate layer (Fig. 11.2) and a vegetation layer comprising different dune plant species (perennial and autochthonous) (Fig. 11.1), where applicable.

Figure 11. General representation of the four layered system, comprising: vegetation layer (11.1), substrate layer (11.2), filter layer (11.3) and expanded cork board (ICB) (11.4) (image of the author).

Experiment I:

Experiment I was monitored from October 2018 until May of 2019 and was composed of 4 treatments. Over the expanded cork board (ICB) and the filter layer it was established a 12 cm thick commercial substrate (Supplier: Landlab - Landl, Lda). The mineral part of the commercial substrate consisted of light expanded clay (2 - 4 mm of diameter) and special volcanic rock (3 – 9 mm of diameter) and the organic part included pine bark humus (0 – 15 mm of diameter) and blonde peat (0 - 40 mm of diameter) (for more information see annex II).

Samples of commercial substrate were sent to the Laboratory A2 Análises Químicas, Lda., Guimarães to analyze substrate characteristics: pH (H_2O), pH ($CaCl_2$), need of limestone addition, electrical conductivity, organic matter, organic carbon, total nitrogen, relation Carbon:Nitrogen and assimilable elements: Phosphorous pentoxide (P_2O_5), Potassium oxide (K_2O).

The plant species tested in the mentioned substrate were: *Ammophila arenaria*, *Corema album* (L.) D. Don and *Helichrysum italicum*. All of these species are autochthonous from Portugal, having a wide distribution along the Portuguese coast (Annex III shows their geographic distribution through Portugal) and were collected from the sand dunes of

Praia de Moledo, Caminha. They were implemented not only as monoculture, in triplicates, but also as polyculture. Besides, were also established three non-vegetated systems which were used as controls (Fig. 12). The performance evaluation in each treatment was accomplished through visual survey.

Corema album* (L.) D. Don** (common name: Camarinha) (Fam. Ericaceae) is a wild, perennial shrub capable to reach 1 m wide. The woody roots are thick and spreading. Besides rare exceptions, where hermaphrodite inflorescences grow, they are dioecious plants (Zunzunegui *et al.*, 2006). They are wind-pollinated and are present in areas with a wide climatic gradient across the Atlantic coast of the Iberian Peninsula, growing in coastal areas (sand dunes, rocky sites and cliffs) from Gibraltar to the North Galicia (Valdés, 1987). Furthermore, although the loss of natural ecosystems and socioeconomic changes caused a decrease on its berry consumption, the white berry presents high potential because of its high nutritional value and serve as food for mammals and birds (León-González *et al.*, 2013; Oliveira and Dale, 2012). ***Ammophila arenaria (Fam. Poaceae) is a perennial grass plant which grows in dense tuffs up to approximately 150 cm. The roots are short and fibrous and together with the rhizome (along which new shoots appear) they assure sand-fixing in dunes and water provision. The leaves show a rolling habit, contain parallel veins and the flowers are hermaphrodite (Chergui *et al.*, 2017; Deysson, 1978). It grows in the foredunes of Europe and North Africa, characterized by low nutrient content, low organic matter and strong environmental stresses (Tutin*, et al.,* 1980; Jebali *et al.*, 2017). Nevertheless, studies as the one of Dalton and collaborators (2004) suggest, that nitrogen fixing bacteria may appear in the rhizome, potentially contributing to its nitrogen nutrition. This underlines the potential of this grass to green roofs, where dinitrogen can become a limiting factor through the years (Skabelund *et al.*, 2015). ***Helichrysum italicum*** (Fam. Asteraceae) is an ecotype of the genus *Helichrysum*, with the ability to grow at a wide range of altitudes, in sandy and rocky areas of the Mediterranean regions. They grow 10 – 30 cm high and are xerophytes[8] and aromatic shrubs. The tubular and yellow flowers have a strong smell similar to curry. Their secretions are endowed with various biological activities with medicinal properties. In the inflorescence the marginal flowers are female and those of the disk are hermaphrodite. The leaves are narrowly linear, silver-green and pubescent (Viegas *et al.*, 2014; Ivanovic *et al.*, 2011; Araújo, n.d.; Diversidade vegetal, n.d.).

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[8] Xerophyte are plants adapted to survive in dry environments - with low or no water availability.
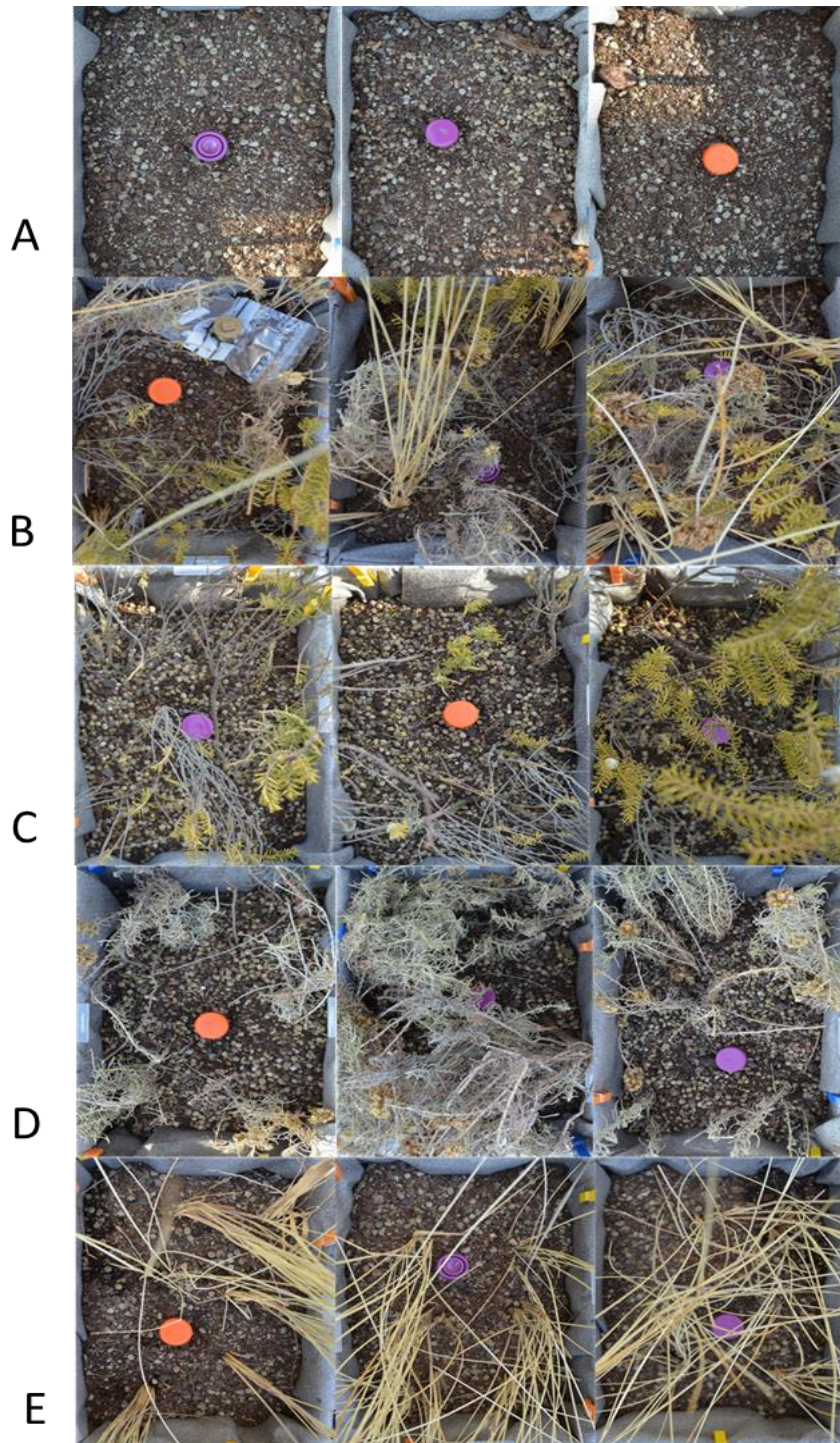
Figure 12. (A) Replicas of commercial substrate without plants (control); (B) Replicas of commercial substrate with polyculture of A. arenaria, C. album and H. italicum; (C) Replicas of commercial substrate with C. album; (D) Replicas of commercial substrate with H. italicum; (E) Replicas of commercial substrate with A. arenaria. Colorful objects correspond to pitfall traps.

Experiment II:

Experiment II was monitored from December 2018 until May of 2019. Over the expanded cork board (ICB) and the filter layer, were tested 12 cm thick substrates: an experimental and a commercial.

The composition of the commercial substrate (Supplier: Landlab - Landl, Lda) (a set of three replicates) was the same referred on Experiment I (details in annex II).

The experimental substrate (a set of three replicas) was created in order to decrease the percentage of organic matter. This allowed the increase of mineral components percentage, and this way, to approach to the sand dune conditions where the selected plant species live. The mineral part of the substrate consisted of 10 % light expanded clay (2 - 4 mm of diameter) (Supplier: Argex – Argila Expandida, S.A.), 20 % light expanded clay (0 – 2 mm of diameter) (Supplier: Argex – Argila Expandida, S.A.) and 40 % sand (Supplier: AREIPOR – Areias Portuguesas, S.A.). The remaining 30 % corresponded to the organic part consisting on a universal substrate (ECO®grow) composed of forest waste humus and blonde peat with organic fertilization (for more information see annex IV). Table 1 summarizes the composition of the selected substrates. Samples of both substrates were sent to the Laboratory A2 Análises Químicas, Lda, Guimarães. to analyze substrate characteristics: $pH(H_2O)$, $pH(CaCl_2)$, electrical conductivity (EC), organic matter, organic carbon, total nitrogen, Relation Carbon:Nitrogen, assimilable elements: Phosphorous pentoxide (P_2O_5) and potassium oxide (K_2O).

Table 1. Composition of the selected substrates.

	EXPERIMENTAL SUBSTRATE	COMMERCIAL SUBSTRATE
ORGANIC PART	*Forest Waste Humus* *Blonde Peat*	*Pine bark humus (0 -15 mm)* *Blonde peat (0 – 40 mm)*
MINERAL PART	*Expanded clay (0 – 4 mm)* *Expanded clay (0 – 2 mm)* *Sand*	*Expanded clay (2 – 4 mm)* *Volcanic rock (3 – 9 mm)*

The plant species tested in the mentioned substrates were: *Calystegia soldanella, Euphorbia paralias* and *Medicago marina,* implemented as polyculture (Fig.13). All these species are autochthonous from Portugal, have a wide distribution along the Portuguese coast (Annex V shows their geographic distribution through Portugal) and were got from the sand dunes of Praia de Francemar, Vila Nova de Gaia, Portugal. ***Calystegia***

soldanella (Fam. Convolvulaceae) is a perennial rhizomatous geophyte herb, with stem up to 50-100 cm and with a shallow spreading habit. The flowers are solitary and in bell-shaped (3 – 5 cm long). The species reproduce by crosspollination through insect-pollination and live on coastal sand foredunes, in many temperate zones of the world (Daniela *et al.*, 2009; Ushimaru and Kikuzawa, 1999). Besides having the capability to restore sand dunes and in erosion control, it has long been used as an edible and medicinal herb, exhibiting various biological activities with high biomedical and biotechnological interest (Ko, 2004; Lee *et al.*, 2017). ***Euphorbia paralias*** (Fam. Euphorbiaceae) is a semi-succulent plant (Tackholm, 1974), having been reported the CAM photosynthetic pathway (Elhaak *et al.*, 1997). It is a perennial and monoecious plant, that grows in dense tufts and can grow up to approximately 70 cm. The roots are long and associated to a woody base where the stems branch grow. The leaves are obovate-oblong in the base, elliptic-oblong in the middle and ovate in the upper parts. The cupped flowers (2 - 5 mm in diameter) are in clusters and flourish in late spring and summer until autumn. Seed dispersal occurs by explosive opening of three-valved fruits capable to reach 2 m of distance. This plant species has a strong relation with insect species such as the case of *hyles euphorbiae* (*Lepidoptera*) larvae which can feed on its leaves, showing potential to attract beneficial insects. The species can be found on sandy seashores along the coasts of South and West Europe (Daniela *et al.*, 2014; Traveset and Navarro, 2017). ***Medicago marina*** (Fam. Fabaceae) is a perennial legume widely distributed along coastal sand dunes from Europe, including the Mediterranean region, to the north of Africa up to mid-Asia. It is completely covered with trichomes, the woody stems grow below the sand and the vegetative branches are rising or erect. The leaves have obovate, denticulate and cuneate leaflets. The golden-yellow flowers are hermaphrodites (Flamini *et al.*, 2003). Furthermore, the *Fabaceae* family is unique for its common ability to establish symbiotic relationships with rhizobia bacteria (Faria *et al.*, 1989). Underlining this, the studies of Alías-Villegas and collaborators (2015) showed the capacity of *Medicago marina* to establish this symbiosis with nitrogen-fixing bacteria. As mentioned before, this can provide a potential value to the nitrogen cycling in green roofs substrate when selecting this plant and consequent bigger adaption capacity of plants.

Figure 13. Replicas of experimental substrate with M. marina, C. soldanella and E paralias (A); Replicas of commercial substrate with M. marina, C. soldanella and E. paralias (B). Colorful objects correspond to pitfall traps.

2.3 Climate and microclimate conditions monitorization

Data of climate conditions were obtained every week through the IPMA website from the closest meteorological station: Pedras Rubras (41° 14' 22.92'' N, 8° 39' 59.076'' W) from December 2018 to May 2019. The registered parameters include precipitation (mm) and maximum and minimum: relative humidity (%), temperature (ºC), wind intensity (km/h) and pressure (hPa).

Microclimate conditions were recorded in experiment I and II through a data logger - iButton HOBO Pendant MX Logger (MX2202) established in the vicinity of the experiments. It recorded temperature data every hour with an accuracy of ±0.5°C from -20° to +70°C from 22 of January to 22 of May 2019. Data were downloaded through HOBOmobile® (version 1.9.4). Furthermore, systems were equipped with a temperature logger DS1922L iButton® installed at the maximum substrate depth in order to register temperature variations. Data were recorded every hour with an accuracy of ±0.5°C from -40° to +85°C from 22 of January to 22 of May. Data were collected from loggers using the software 1-WIRE®/IBUTTON®.

2.4 Macrofauna monitorization

According to Richter and collaborators (2013) protocol, pitfall traps were setup in all systems. Pitfall trapping, used in order to characterize soil macrofauna[9], is one of the most widely used methods for studies of species occurrence. It enables to compare abundance in different habitats, to examine spatial distribution patterns, to study daily activity rhythms and community surveys (Richter *et al.*, 2013). It assumes that once the target organisms fall, they cannot go out and they will not actively avoid falling into the trap. This method has been used successfully for surface-active organisms, which have a phase of their life cycle on the ground and for comparing the incidence of surface activity of species on a diurnal or seasonal basis, or in relation to weather patterns (Silva and Alves, 2013).

One pitfall trap was placed on the surface of each container taking in consideration the dimension and shape of the area. Each trap consisted on a 50 mL Falcon Conical Centrifuge Tubes with 3 cm of diameter and 11 cm of depth, cover protected from the rain, buried in the ground with a gap at the ground surface level, allowing the fall of the fauna. Macrofauna data was collected every 10 days, during the whole experimental

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[9] Fauna with a size bigger than 2 mm.

time. This frequency time allows the reduction of the interference in the microhabitat and consequently traps avoidance by the invertebrates. After bottle removal from the ground, invertebrates were collected, observed through magnifying glass (OLYMPUS SZX10) and preserved in ethanol 70%. Posterior generic taxonomic identification based on the morphological characterization was accomplished through Dahms, *et al* (1979), allowing the characterization of local fauna.

2.5 Physiological measurements of plants

In order to make accurate and quick measurements of key photosynthetic parameters, light saturation curve measurements were accomplished on the adaxial leaf surface using a portable Pulse-Amplitude-Modulation (PAM) fluorometer (JUNIOR-PAM chlorophyll fluorometer; Walz, Effeltrich, Germany). Measurements were conducted in the field during the growing season (spring) on cloudless days and were performed between 10 a.m and 1 p.m. according to the manufacturer indications (Heinz Walz GmbH, 2007). Samples were dark acclimated with a black cloth during at least 20 minutes. Dark adaptation allows photosystem II (PSII) reaction centers, present in the thylakoid to open and electron transport chain to be oxidized (Genty *et al.*, 1989). After that, 12 levels of consecutively increasing intensities of actinic illumination take place, 1 mm distanced from the leaf and with the corresponding PAR (Photosynthetic active radiation) of 1500 µmol photons·m^{-2}·s^{-1}). Physiological variables (ETRmax, α and Fv/Fm) were calculated using the WinControl-3.28 software (Heinz Walz Gmbh, Effeltrich, Germany). The measure of "Rapid Light Curves" (RLC) in the software provide information on the current state of photosynthesis. Between the key parameters we selected the initial slope of the curve (Alpha, α), related to the maximum photosynthetic efficiency and the ETR$_m$ (µmol electrons**/**(m · s)) corresponding to the maximum electron transport rate on the electron transport chain. Photochemical efficiency of PSII (Fv/Fm), which is present in the photosynthetic membrane, is also evaluated as a measure of the health of the photosystems and consequently an indirect measure of plant stress. Fv (mv) corresponds to the variable fluorescence as the difference between Fm and F0 (Fm - F0). Fluorescence detected in the dark is designated as F0 (mv). F0, or minimal fluorescence corresponds to the intrinsic fluorescence from the antenna of fully oxidized photosystem II (PSII). Maximum fluorescence, resulting from fully reduced PSII reaction center after light emission is designated as Fm (mv) (Schreiber, 2004).

Physiological measurements were conducted in experiment II to evaluate plant performance in the selected systems. For each selected plant were carried out three measurements, which were used for statistical analysis. Statistical analysis was performed through IBM SPSS 26 software package.

2.6 Water quality characterization

In experiment I and II, samples of water runoff from each container and precipitation samples were analyzed. Water quality was evaluated through various parameters, namely pH, electrical conductivity (EC), chemical oxygen demand (COD) and nutrients (phosphates, nitrates and ammonium), from multiple rainfall events within the experimental period.

Water runoff from each container (corresponding to the total accumulated water runoff – designated as cumulative samples), was analyzed directly at the local within 96h hours of the precipitation event for pH and electrical conductivity (EC) through a portable multi meter (pHenomenal® MU 6100 H; VWR, Leuven, Germany). Before the analysis with the portable multi meter, water samples were collected to nutrient analysis through a syringe and filtered through a syringe filter holder with a 0.7 µm pore size glass microfiber filter. COD samples were collected directly from the container without filtering. All collected samples were stored at $-20\,^{\circ}C$ until further analysis. Statistical analysis was performed through IBM SPSS 26 software package.

Chemical oxygen demand (COD) is a measure of the organic matter quantity as the amount of dissolved oxygen required to cause chemical oxidation of the organic material (Patil *et al.*, 2012). It was measured using a reagent kit HI 93754B-25 MR: COD Medium Range (0 to 1500 mg/L) adapted from EPA 410.4 colorimetric method. The method consists on the reduction of dichromate ion to the chromic ion by oxidizable organic compounds at $150\,^{\circ}C$ for 2 hours.

2.6.1 Nutrient analysis

Nitrite ($N\text{-}NO_2^-$), ammonium ($N\text{-}NH_4^+$) and phosphates ($P\text{-}PO_4^{3-}$) concentrations were quantified as described in Grasshoff *et al.* (1983) and nitrates ($N\text{-}NO_3^-$) by the method of Jones (1984). Sample dilution was accomplished when necessary and each sample was analyzed in triplicate. For the quantification, aqueous standard solutions were done for the respective nutrient, assuming a linear response. Samples were read on the

spectrophotometer in the respective wavelength. From the calibration curve, we calculated the corresponding detection limit and the nutrient concentrations in mg/L.

Dissolved inorganic phosphate (commonly referred to as orthophosphate) was measured using the ascorbic acid method of Murphy and Riley (1962) (Grasshoff *et al.*, 1983). After addition of ascorbic acid and mixed reagent to the sample, they react rapidly with phosphate ions and create a bluish complex. The absorbance was read in the spectrophotometer after 10-30 minutes of incubation at a wavelength of 880 nm;

Ammonium was measured using the phenol-hypochlorite reaction by the method of Koroleff (*in* Grasshoff *et al.*, 1983). A blue color arises due to the formation of indophenol, which in turn is resultant from the presence of catalyzers - phenol and hypochlorite. Since our samples had a salinity inferior to 5, it was also added magnesium reagent due to its buffer capacity. The absorbance was read in the spectrophotometer after 6 – 30 hours of incubation at a wavelength of 630 nm;

Nitrites were determined according to Grasshoff *et al* (1983). The method is based on the nitrite reaction, emerging a pink color with intensity proportional to the quantity of nitrite. This is due to the addition of a reagent with sulphanilamide on its composition. In interaction with nitrite, it creates a diazotized compost which in turn binds to N-(1-naftil)-etilenodiamina originating a colorful complex. The absorbance was read in the spectrophotometer after 10 – 30 minutes of incubation at a wavelength of 540 nm;

Nitrate concentration was determined using the method of Jones (1984). The method lays on the nitrate reduction to nitrites. This occurs due to the contact of the sample with spongy cadmium in the presence of a buffer solution of ammonium chloride (NH_4Cl). After 1:30 hours under constant shaking at room temperature nitrites are colored with nitrite reagent. The absorbance was read in the spectrophotometer after 10 – 30 minutes of incubation at a wavelength of 540 nm. The final NO_3^- concentration was calculated subtracting the concentration of nitrites to de solution of nitrites obtained by the reduction of nitrates.

2.7 Microbial community characterization

2.7.1 Substrate sampling

Substrate samples for molecular analysis in experiment I consisted on the substrate before implementation in the experiment (T0) and after one month, on samples collected every 2 months over a period of 6 months, resulting in four samplings. The T0 sample

characterized the microbial communities present in the selected substrate before its use in the experiment. This provided valuable information of how patterns of microbial diversity (namely Archaea, Bacteria and Eukarya) were shaped, considering time, climate and plant species.

Thus, the four samplings were identified as: sampling M0, M1, M3 and M5, corresponding to the substrate before implementation (M0), sampling in November (M1), sampling in January (M3) and sampling in March (M5). For every treatment, samples were collected in triplicate to form a composite sample (30 cm³). Samples were subsequently homogenized by mixing/shaking in DNAse and RNAse free falcons, divided in two subsamples and stored at -80ºC until further processing and analysis of microbial 16S rRNA amplicons.

Therefore, samples of experiment I corresponded to:

(1)   Substrate before implementation in the experiment (T0);

(2)   Composite substrate samples from the control with no vegetation (C) obtained in November (C-N), January (C-J) and March (C-M);

(3)   Composite rhizosphere samples from the monoculture of *C. album* (CA) obtained in November (CA-N), January (CA-J) and March (CA-M);

(4)   Composite rhizosphere samples from the monoculture of *A. arenaria* (AA) obtained in November (AA-N), January (AA-J) and March (AA-M);

(5)   Composite rhizosphere samples from polyculture (PL) of *C. album* and *A. arenaria* obtained in November (PL-N), January (PL-J) and March (PL-M).

In the end, 13 composite samples were obtained in Experiment I (one treatment excluded due to plant death).

2.7.2 eDNA extraction and quantification

Environmental DNA (eDNA) was extracted from 500 mg (wet weight) of each substrate sample using DNeasy PowerSoil Kit® (Qiagen) following the manufacturer's instructions. Cells were lysed by a combination of chemical agents used in the initial steps and mechanical shaking by power beads.

Extracted eDNA was initially eluted in 100 μl of Elution Solution (a sterile elution buffer of 10 mM Tris (hydroxymethyl) aminomethane) and then concentrated in a final volume of 30 μl. Next was used Qubit dsDNA HS (High Sensitivity) Assay Kit (ThermoFisher Scientific Inc) and DNA concentrations were directly measured using Qubit® 3.0 Fluorometer. Samples were then frozen at -20°C until further processing.

2.7.3 Sample Preparation and Sequencing of SSU rRNA amplicon

eDNA extracted was sent for MiSeq Illumina Next Generation Sequencing. Amplicon libraries were sequenced in Biocant Park, SA and generated by amplifying the hypervariable V4-V5 region of the 16S rRNA gene with the primer pair 515F-Y (5′-GTGYCAGCMGCCGCGGTAA-3′) and 926R (5'-CCGYCAATTYMTTTRAGTTT-3') (Parada, Needham, & Fuhrman, 2016). PCR reactions followed the KAPA HiFi HotStart PCR Kit as stated by the manufacturer suggestions. The sequences were further reamplified in a limited-cycle PCR reaction to add sequencing adapters and indexes to both ends of the amplified target region according to manufacturer's recommendations (Illumina, 2013). PCR products were then one-step purified and normalized using SequalPrep 96-well plate kit (ThermoFisher Scientific, Waltham, USA) (Comeau, Douglas, & Langille, 2017), pooled and pair-end sequenced in the Illumina MiSeq® sequencer with the V3 chemistry, considering the manufacturer's instructions (Illumina, San Diego, CA, USA) at Genoinseq (Cantanhede, Portugal).

Sequence data were processed at Genoinseq (Cantanhede, Portugal). Raw reads were extracted from Illumina MiSeq® System in fastq format and using PRINSEQ version 0.20.4 (Schmieder and Edwards, 2011) they were quality-filtered which allowed the removal of sequencing adapters, trim bases with an average quality lower than Q25 in a window of 5 bases and reads with less than 150 bases. The forward and reverse reads present in the fastq file were merged by overlapping paired-end reads using the AdapterRemoval version 2.1.5 (Schubert, Lindgreen, & Orlando, 2016) considering default parameters.

2.7.4 Bioinformatic analysis

Post sequence processing started using Mothur (version 1.35.1;https://www.mothur.org/) software in order to convert fastaq files to fasta. 16S rRNA gene sequences were then submitted (aligned, quality checked and classified) by SILVAngs automatic software pipeline – (SILVAngs 1.3) (Quast *et al.,* 2013), and processed using SILVAngs default parameters. Quality steps were automatically performed by SILVAngs :reads were aligned using the SILVA Incremental Aligner (SINA SINA v1.2.10 for ARB SVN (revision 21008)) (Pruesse *et al.*, 2012) against the SILVA SSU rRNA SEED and quality controlled (Quast *et al.,* 2013). In the step of quality control, reads that did not follow established prerequisites of minimum length of 50 aligned

nucleotides, ambiguities and homopolymers minor than 2% were excluded from further processing. Putative contaminations and artefacts reads with a low alignment quality were identified and excluded from downstream analysis. After that, identical reads were identified in the dereplication process. The unique reads were clustered (OTUs) and the reference read of each OTU was classified. Dereplication and clustering was accomplished using cd-hitest (version 3.1.2; http://www.bioinformatics.org/cd-hit) (Li and Godzik, 2006) running in accurate mode, ignoring overhangs, and applying identity criteria of 1.00 and 0.98, respectively. The classification process was performed by a local nucleotide BLAST search against the non-redundant version of the SILVA SSU Ref dataset (release 132; http://www.arb-silva.de) using blastn (version 2.2.30+; http://blast.ncbi.nlm.nih.gov/Blast.cgi) with standard settings (Camacho *et al.*, 2009). The classification of each OTU reference read was mapped onto all reads that were assigned to the respective OTU. Reads without any BLAST hits or reads with weak BLAST hits that did not exceed 93%, remain unclassified and assigned to the metagroup "No Relative".

Taxonomic abundance tables at different levels were produced in order to show the total number of sequences, and their corresponding relative abundance, assigned to each taxonomic group within each sample.

2.7.5 Downstream analysis

A summary of sequence processing, rarefaction curves of alpha diversity created in SILVAngs analysis platform and graphs of relative abundance of 16S rRNA genes per sample was accomplished.

Considering the overall 16S microbial communities relative abundance, a distance matrix-based method, namely, non-metric multidimensional scaling (nMDS) according to the Bray Curtis similarity was accomplished to look for patterns of microbial diversity over time. A hierarchical cluster analysis was also performed with PRIMER 6 (version 6.1.13) & PERMANOVA + (version 1.0.3) in order to complete information of nMDS considering the dissimilarity between samples. Species diversity was calculated using the Shannon Index using the same program to assess changes in microbial biodiversity between treatments along time.

2.8 Statistical analysis

Statistical analysis was performed through IBM-SPSS statistic software (v. 26).
When the assumption of normality was confirmed with the Shapiro-Wilk test (rejection level of $\alpha = 0.05$) and the assumption of homogeneity of variances by Levene's test was not rejected test ($p > 0.05$), one-way ANOVA was performed. When significant differences observed ($p < 0.05$), we proceeded to a post-hoc Tuckey test to evaluate between which systems we could find statistically significant differences. On the other hand, when the assumption of normality was not confirmed, we proceeded to the non-parametric test Kruskal-Wallis H test and multiple pairwise allowed to observe statistical differences.

Chapter III

Results and Discussion

3 RESULTS AND DISCUSSION

3.1. Monitoring local conditions

3.1.1 Climate

Data obtained in Pedras Rubras meteorological station by IPMA website, with the purpose to characterize climate conditions, were registered from December 2018 to May 2019. Through data presented in table 2, it's possible to observe that the highest temperature value was obtained in May and the lowest temperature value was obtained in February. Wind values ranged between 87.1 and 6.5 km/h considering all data. December was the month that presented higher precipitation average, followed by April, January, March, February and May. Maximum relative humidity was similar in all months, ranging between 9 6% and 100 %. Minimum relative humidity ranged between 12 % and 26 %. Pressure values ranged between 998.3 and 1034.4 hPa.

Table 2. Climate data characterization registered from Pedras Rubras meteorological station. Data include maximum and minimum temperature (TºC), wind (km/h) and relative humidity (R.H.) (%), pressure (hPa) and average precipitation (mm) from December to May.

	T (ºC)		Wind (km/h)		Precipitation (mm)	R.H. (%)		Pressure (hPa)	
	max	min	max	min	Average	max	min	max	min
December	19.7	6.9	85.3	6.8	4.4	98	26	1032.8	1016.7
January	18.4	1.5	74.5	7.9	2.5	96	17	1034.4	1002.1
February	24.1	1.4	79.9	7.2	1.2	96	21	1031.2	998.3
March	25.3	3.3	87.1	6.5	2.2	98	13	1033.2	1006.6
April	27.1	4.8	68.0	8.6	4.2	98	23	1027.8	999.2
May	32.3	8.0	60.8	8.6	0.2	100	12	1022.5	1011.6

3.1.2 Fauna monitorization

Various arthropods were registered from pitfall traps (Fig.14). Macrofauna was collected rapidly after systems implementation in experiment I but not in experiment II (Table 3). This may be due to the occurrence of frequent rain events after experiment II implementation that led to an increase of litter in samples and complicate fauna extraction. In experiment I were found organism of Araneae, Formicidae, Lepidoptera, Coleoptera, Culicidae, Hemiptera, Diplopoda and Coccinellidae and in experiment II were found organisms of Aranae, Culicidae and Orthoptera. Our results indicate that the implementation of a green roof could potentially provide habitat for various arthropods. Just as proposed by Mayrand and Clergeau (2018) limited patch size and distinct habitat conditions at the building were considered limitations to species richness and diversity. Our results are in accordance with Ksiazek-mikenas and collaborators (2018) where Araneae and Hemiptera were between the most abundant organisms. Studies of longer duration are necessary in order to conclude if green roofs are capable to support high biological diversity for various generations (Ksiazek-mikenas *et al*., 2018).
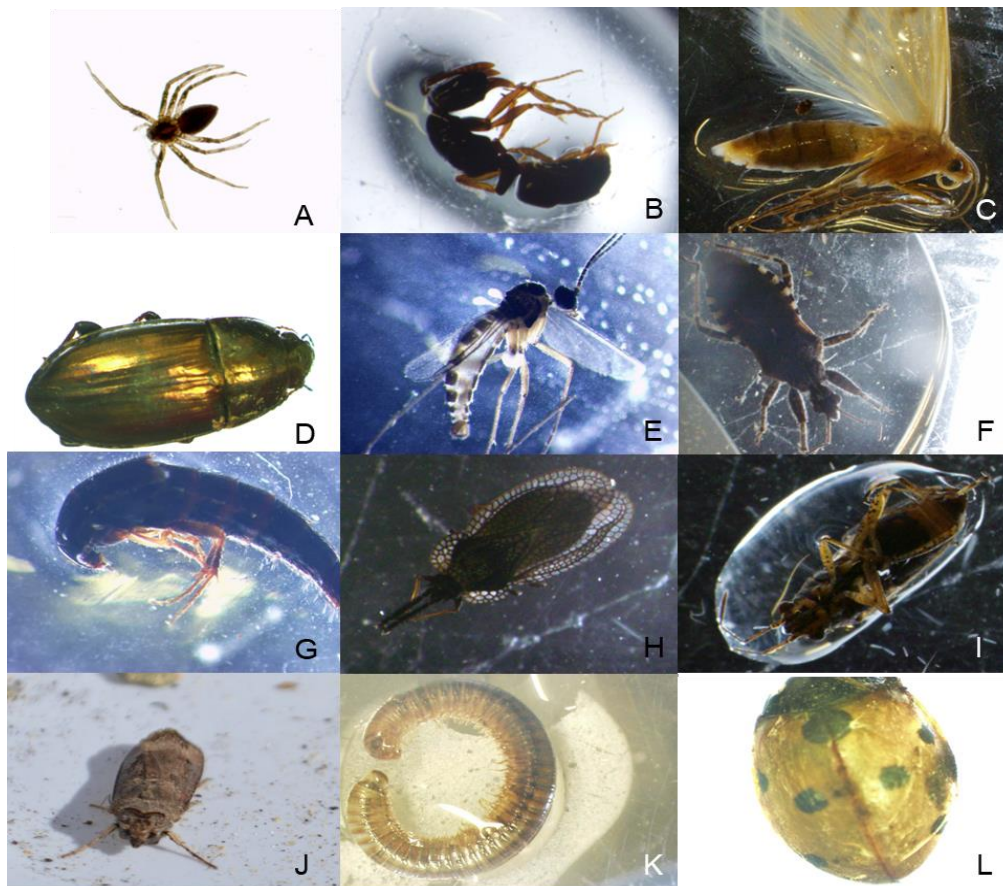
Figure 14. Macrofauna found in pitfall traps include organisms from the groups of Araneae (A), Formicidae (B), Lepidoptera (C), Coleoptera (D), Culicidae (E), Hemiptera (F, G, H, I), Orthoptera (J), Diplopoda (K) and *Coccinellidae (L).*

Table 3. Macrofauna collected during the whole experimental time considering experiment I and II (C: Control, CA: Corema album, HI: Helichrysum italicum, AA: Ammophila arenaria and PL: Polyculture treatments in experiment I; E: Experimental substrate and CM: Commercial substrate in experiment II).

Groups	October	November	December	January	February	March	April	May
Araneae	CA; AA;	AA;					AA;PL;	AA; E;
Coccinellidae								CA;
Coleoptera							C;	
Culicidae						CM;	PL;	PL; C;
Diplopoda		CA;						
Formicidae	PL;							
Hemiptera		C;	HI;			AA;		
Lepidoptera	AA;							
Orthoptera	HI;					E;		

3.2 Performance comparison between different plants as mono and polyculture and a control under the same substrate

3.2.1 Plant establishment

Considering the selected plants (*A. arenaria, C. album* and *H. italicum) A. arenaria* was the plant that presented better survival capacity, being capable to regenerate. Although the aerial part of *C. album* did not show any development, were observed new roots growing during the spring season. Previous studies showed good survival capacity of *H. italicum (e.g.* Papafotiou *et al.*, 2013; Monteiro *et al.*, 2017b). However, in our study conditions*, H. italicum* did not thrive, having been observed the development of fungus on various plant roots. One possible explanation for this may be that, being implemented on autumn season, with rainy days and the fact that substrates with high organic matter retain high quantities of water, we can consider that the plant received more water and organic compounds than it can support and it's used on its natural environment.

Scientific literature couldn't be found regarding the use of *A. arenaria* in green roofs although it is known that it was already used by the industry, as we can see in Landlab website (Landlab, n.a.). To our knowledge, there is no literature of *C. album* being used in green roofs experiments.

3.2.2 Substrate characterization

The commercial substrate characterization showed an acidic nature (pH < 7), high electrical conductivity (EC) and high content of: organic matter, organic carbon, total nitrogen, phosphorous pentoxide, potassium oxide and high ratio of carbon:nitrogen (C:N). Table 4 summarizes the results of the selected substrate, considering parameter and method of analysis.

Table 4. Summary of commercial substrate characteristics.

Parameter	Method	Units	Commercial substrate
pH (H$_2$O)	ISO 10390:2005 / Potentiometry	pH Units	5.84 ± 0.01
pH (CaCl$_2$)	ISO 10390:2005 / Potentiometry	pH Units	5.56 ± 0.01
EC	ISO 11265:1994 / Conductometry	µS/cm	1617 ± 2
Organic matter	Calculus (M.O. = Corg x 1,724)	%	46.13 ± 0.01
Organic carbon	prNE 15936:2009 / Conductometry	%	26.76 ± 0.04
Total nitrogen	NE 13654-2:2001 / Conductometry	%	0.57 ± 0.02
Ratio C:N	Calculus	%	47.2
Phosphorous pentoxide (P$_2$O$_5$)	ISO 22036:2008 / Mehlich extraction 3	mg/kg	139.3 ± 0.4
Potassium oxide (K$_2$O)	ISO 22036:2008 / Mehlich extraction 3	mg/kg	1595.2 ± 1.3

3.2.3 Microbial community characterization

This section is reserved to evaluate the existence of differences on microbial communities diversity and structure between the tested treatments (substrate before implementation (T0), vegetated and non-vegetated systems) and compare the microbial communities before implementation (T0) and over a six-month period. Therefore, the variables in analysis correspond to time and plants.

3.2.3.1 Taxonomy of microbial communities at the substrate and rhizosphere level

Through SILVAngs pipeline, 696385 sequences were generated. After removal of 101225 sequences with low-quality (14.54 % of the total dataset), a total of 581065 (83.44 %) sequences were generated from 13 samples. From those classified sequences, 14095 (2.02%) were considered as unknown phylum ("no relative").

The taxonomic characterization of the substrate and rhizosphere microbial communities obtained by 16S rRNA gene sequencing, detected taxa belonging to the three domains: Archaea, Bacteria and Eukarya.

A total of 57 phylum were identified in all samples. Among them, the 10 dominant phylum, showed in Figure 15, represent 93.26 % of the total sequencing and have a relative abundance superior to 1 %.

Considering all treatments and sampling dates, *Proteobacteria* was the most abundant phylum detected in our dataset (with relative abundance between 39.55 – 53.93 %). It was followed by *Bacteroidetes* (9.73 – 18.84 %), *Actinobacteria* (6.05 – 13.19 %), *Planctomycetes* (5.01 – 7.48 %), *Acidobacteria* (4.44 – 7.36 %), *Chloroflexi* (2.80 – 7.80 %), *Verrucomicrobia* (3.05 – 5.57 %), *Opisthokonta* (1.13 – 3.33 %), *SAR*[10] (0.45 – 3.54 %) and *Gemmatimonadetes* (0.81 – 1.87 %). No substantial differences were observed between the different treatments and over time regarding those phylum (Fig. 15). This can underline that the substrate used was the main driver of phylum communities abundance. Our results are in accordance with the ones of Mitchell (2017) PhD thesis results, which studied Midwestern U.S. green roofs ecosystems through various years. Just as presented in our results, *Proteobacteria, Actinobacteria, Acidobacteria, Chloroflexi, Gemmatimonadetes* were between the seven most abundant phylum. Furthermore, phylum found on our study, namely *Proteobacteria*, *Acidobacteria, Verrucomocrobia, Actinobacteria, Bacteroidetes, Chloroflexi,* and *Gemmatimonadetes* are generally known as dominant phylum in soil libraries (Janssen, 2006) demonstrating that artificial substrates have similar microbial biodiversity of natural soils.

Just as Mitchell and collaborators (2018) study in green roofs microbiome, the most abundant archaeal 16S rRNA gene sequences corresponded to *Thaumarchaeota* (50.50 % of total archeal sequences), which in turn were followed by *Nanoarchaeaeota* and *Euryarchaeota* with less abundance.

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[10] SAR is an acronym of the groups Stramenopiles, Alveolates, and Rhizaria
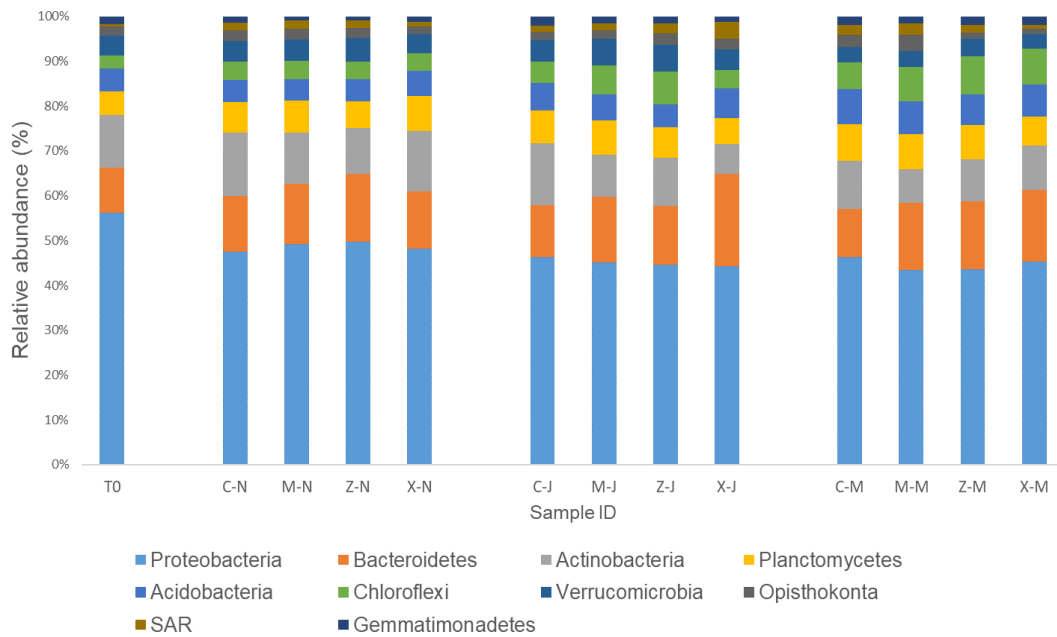
Figure 15. Relative abundance (%) of 16s rRNA gene sequences of the 10 dominant phylum with relative abundance superior to 1% across all treatments over time (T0: substrate before implementation, C-N: Control in November, CA-N: C. album in November, AA-N A. arenaria in November, PL-N: Polyculture in November, C-J: Control in January, CA-J: C. album in January, AA-J: A. arenaria in January, PL-J: polyculture in January, C-M: Control in March, CA-M: C. album in March, AA-M: A. arenaria in March, PL-M: Polyculture in March).

3.2.3.2. Alpha-diversity analysis

Alpha diversity rarefaction curves (Fig. 16), provides information about the sequencing coverage in each sample based on the number of OTU registered. The results show that the sequence coverage was not sufficient to represent all taxa present in and on concrete since the curves have not reached a plateau.
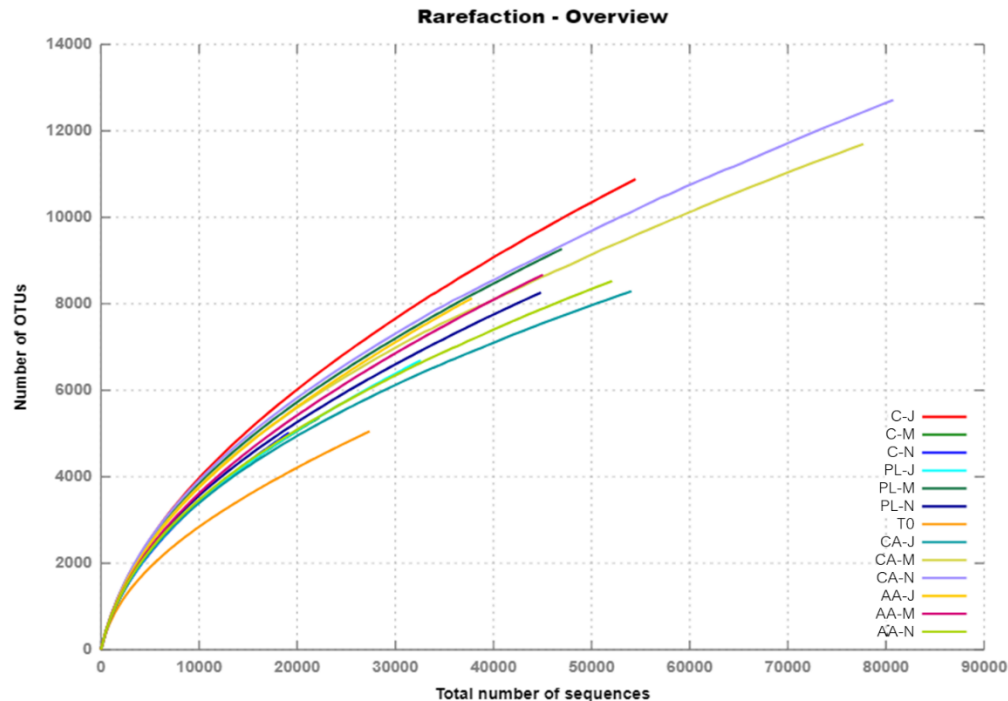
Figure 16. Rarefaction curve of microbial richness from the 13 samples considering time and treatments. (T0: substrate before implementation, C-N: Control in November, CA-N: C. album in November, AA-N A. arenaria in November, PL-N: Polyculture in November, C-J: Control in January, CA-J: C. album in January, AA-J: A. arenaria in January, PL-J: Polyculture in January, C-M: Control in March, CA-M: C. album in March, AA-M: A. arenaria in March, PL-M: Polyculture in March).

Shannon index, an α-diversity index, allowed us to quantify the diversity of species within a dataset. The results shown in Table 5, allowed to infer that, as expected, T0 corresponded to the treatment with lower diversity value (5.097), representing the starting point of all treatments in study. The Shannon index values for the treatments ranged from: 5.276 – 5.289 to the Control, 5.166 – 5.266 to C. album, 5.243 – 5.388 to *A. arenaria* and 5-254 – 5.315 to Polyculture. After plants implementation, it would be expected a considerable increase in microbial biodiversity, as a result of plant exudates that attract bacteria. In fact, the highest diversity results obtained, corresponded to *A. arenaria* and Polyculture. However, no considerable differences were observed between all treatments regarding this index. This highlights the influence of the substrate and possibly, climate characteristics on microbiome communities.

Table 5. Summary of number of sequences classified, richness and diversity of the different treatments over time. (T0: substrate before implementation, C-N: Control in November, CA-N: C. album in November, AA-N A. arenaria in November, PL-N: Polyculture in November, C-J: Control in January, CA-J: C. album in January, AA-J: A. arenaria in January, PL-J: Polyculture in January, C-M: Control in March, CA-M: C. album in March, AA-M: A. arenaria in March, PL-M: Polyculture in March).

Treatments	Number of sequences classified	Richness[1]	Diversity[2]
T0	16886	5051	5.097
C-N	11756	5019	5.289
CA-N	46957	12715	5.266
AA-N	30829	8528	5.252
PL-N	27181	8261	5.315
C-J	32395	10882	5.276
CA-J	33254	8290	5.166
AA-J	23224	8129	5.388
PL-J	18744	6693	5.254
C-M	14605	5371	5.287
CA-M	41983	11695	5.182
AA-M	25066	8673	5.243
PL-M	29160	9268	5.300

NOTE: [1]NUMBER OF OTUS CLASSIFIED; [2]SHANNON DIVERSITY INDEX (H')

3.2.3.3 Beta-diversity analysis

Through Non-metric Multi-Dimensional Scaling (nMDS) (Fig. 17), a representation of community patterns based on similarity was obtained. The results showed that there is a temporal difference between all sampling dates. As expected, it is observed a bigger dissimilarity in species composition between the first sampling date (M1) and the third sampling date (M5) since it ranges from autumn (M1) to winter (M3) and finally spring (M5). Furthermore, the results showed a distance considering microbial communities composition between treatments. This indicate that the differences were mostly determined by abundance patterns of rare taxa. Comparing the first sampling date (M1) and the second sampling date (M3), was observed a proximity in microbial communities between the control of November, January and for *C. album* in November. Although it was observed late root development in *C. album*, it presented very low development in the initial months of the experiment. This may be the cause for that proximity with the Control. Furthermore, similarities between the treatments *A. arenaria* and polyculture

were observed mainly in the first two sampling dates of the experimental time. This is possibly because in the polyculture, where three plant species were established, only *A. arenaria* presented good survival capacity. The increased distance between them in the third sampling date (M5), may be associated with plant performance and/or season.
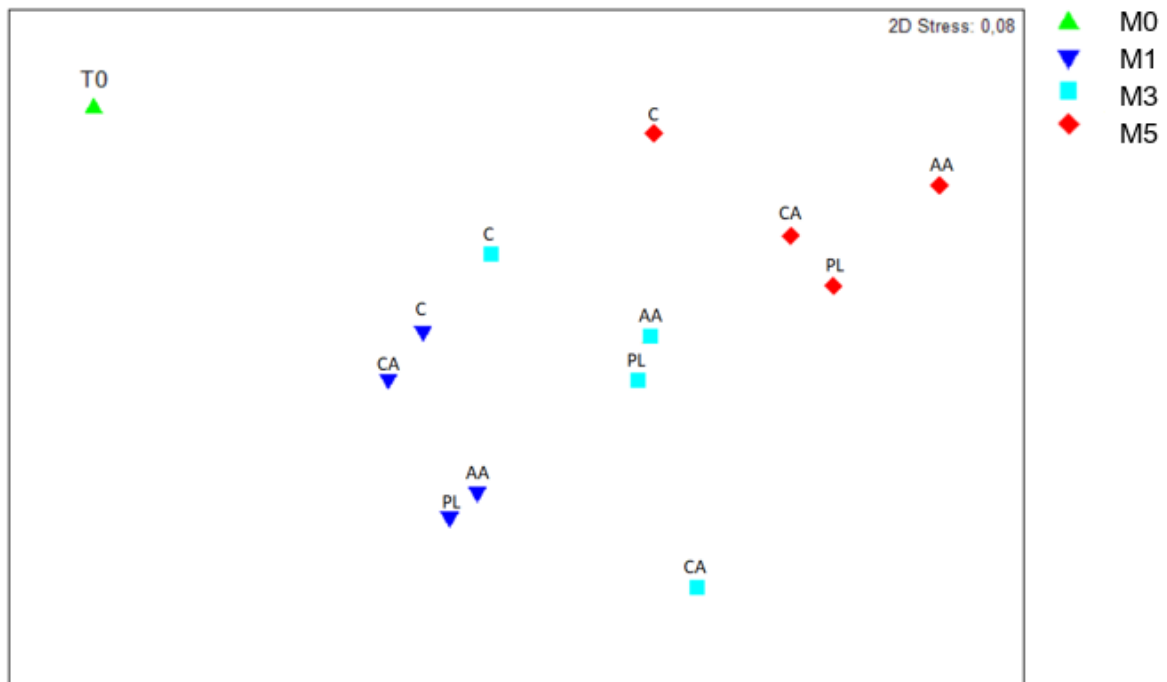
Figure 17. Non-metric multi-dimensional scaling (nMDS) based on Bray Curtis similarity considering the treatments in analysis (C: Control, CA: C. album, AA: A. arenaria, PL: Polyculture) and sampling dates. (M0: substrate before implementation (T0), M1: first sampling date in November, M3: second sampling date in January, M5: third sampling date in March).

A dendrogram (Fig. 18) was also accomplished to underline the above results. The hierarchical clustering analysis of 16S rRNA gene sequences allowed to observe a clearer pattern considering the similarities between treatments. Here, we can highlight the closest similarities in species composition between the Control in November and in January, having both a close similarity with *C. album* in November. Furthermore, we can also see close similarities between Polyculture in March, *C. album* in March and *A. Arenaria* in March and between the first (M1) and second (M3) sampling dates. The closest similarity between Polyculture and *C. album* in March can be possibly explained by the root development of *C. album*.
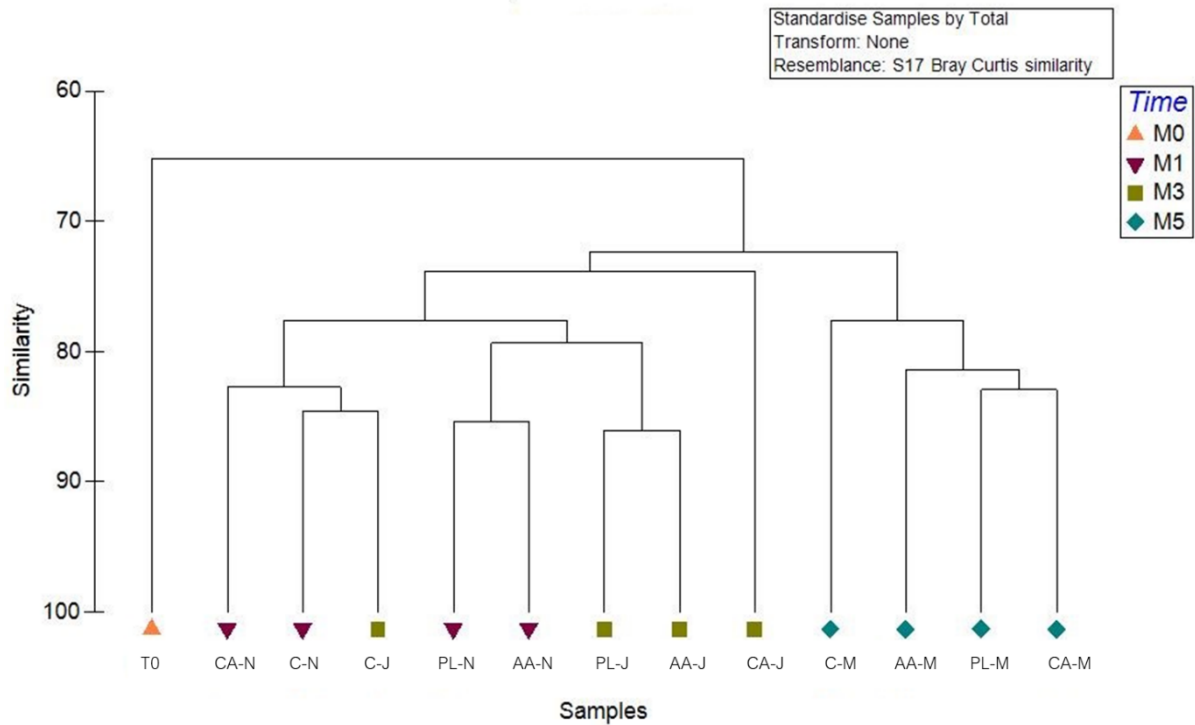
Figure 18. Cluster analysis based on Bray Curtis similarity considering the treatments in analysis and sampling dates. (M0: substrate before implementation (T0), M1: C-N: Control in November, CA-N: C. album in November, AA-N A. arenaria in November, PL-N: Polyculture in November, M3: C-J: Control in January, CA-J: C. album in January, AA-J: A. arenaria in January, PL-J: Polyculture in January, M3: C-M: Control in March, CA-M: C. album in March, AA-M: A. arenaria in March, PL-M: Polyculture in March).

The results of microbial community characterization gave us important information regarding microbial communities changes. Our main objectives with this characterization were thereafter to (i) analyze if monoculture-type and polyculture-type plants implemented in the same substrate-type differ in their rhizosphere microbiome (ii) to analyze if there were significant differences between unvegetated and vegetated systems and finally (iii) to analyze temporal changes in substrate and rhizosphere microbiome. Overall, we found distinct patterns between sampling dates, concluding that substrate microbial composition changes with time and is likely to be affected by seasonality. Moreover, we were capable to see that plant implementation did influence substrate microbiome diversity. The results presented (ii) are in accordance with the ones of Schmid and collaborators (2018), that observed by 16S rRNA gene sequencing, significant influences by different plant species identity in microbiomes composition at the rhizosphere. Further studies would be necessary to analyze the influence in substrate microbiome by monoculture versus polycultures, and thereafter, conclude about their ecological functioning at the rhizosphere level.

3.2.3.4 Prominent genera associated to *A. arenaria*

The focus on this section relies on the substrate microbiome before implementation (T0), in the non-vegetated control and the rhizosphere microbiome in monoculture and polyculture forms *of Ammophila arenaria* over time. The emphasis on *A. arenaria* is due to the fact that it was the plant that presented better performance, and its microbiome has been a focus of study for several years.

Although we obtained various bacteria genera associated to *A. arenaria* described in previous studies such as: *Allorizhobium, Ochrobactrum sp., Pantoea, Pseudomonas, Sphingomonas, Stenotrophomonas, Roseomonas* (Dalton, 2004); *Azotobacter, Pseudomonas, Lactobacillus, Sporosarcina, Flavobacterium, Microbacterium, Acinetobacter, Azospirillum, Serratia, Bacillus, Microbacterium, Mycobacterium, Clostridium, Pseudomonas, Arthrobacter, Acinetobacter* (Ruppel, 1989), most of them were also present in the substrate used (T0). This causes some controversy about the real influence of *A. arenaria*. However, regarding *Pseudomonas* and *Arthrobacter,* although there was a small abundance in T0, the values increased exponentially in the last sampling date (M5).

Between all identified genera, *Clostridium, Azotobacter* and *Azospirillum* where not present in T0, however, none of them presented high abundance values. All of them are known to be involved in nitrogen fixation (Wagner, 2011). Furthermore, one OTU of the genera *Rhizobium* was identified. Interestingly, considering the whole sampling dates and T0, it was only found in the last sampling date (M5) - in the monoculture and polyculture treatments. These genera are widely known by their capacity to establish nitrogen-fixing symbiosis with leguminous plants. Hereupon, the presence of nitrogen fixing microorganisms presents high potential to substrate fertility and therefore, to plant development (Mitchell *et al.*, 2018). Further studies would be required to determine their functional gene diversity and their metabolic activity on those substrates.

3.2.4 Water quality

This section is reserved to evaluate how water runoff quality parameters changed over time (samplings S1-8) and infer about the presence or absence of significant differences between the tested treatments (vegetated, non-vegetated systems and rainwater). Thereafter we could infer about the influence of the green roof components in rainwater composition that percolates them.

Data from *H. italicum* were only acquired from S1-S5 due to plant death and were not included in the statistical analysis, being considered only the Control, *C. album*, *A. Arenaria* and Polyculture.

3.2.4.1 pH

Considering pH values along time (S1-8) (Fig. 19), little variations were observed in all data of vegetated and non-vegetated systems. Considering all sampling dates, the values ranged from 6.2 to 7.7 (see table 6).

No statistically significant differences ($p > 0.05$) were observed between the tested systems (Control, Polyculture, *C. album* and *A. arenaria*), concluding that there was no influence by the different selected species as mono and polyculture when comparing to the non-vegetated Control. Considering this, our results are in accordance with the study of Monteiro and collaborators (2017a) but in opposition with the study of Beecham and Razzaghmanesh (2015) where vegetated and non-vegetated roofs had different influence on pH values. Further studies considering time and vegetation density are necessary to evaluate the potential influence of the selected vegetation in rainwater pH. Additionally, statistical analysis showed a significant difference ($p < 0.05$) between the tested systems and rainwater. Consistent with previous studies (e.g. Beecham and Razzaghmanesh, 2015) the mean pH values were lower in the tested systems when compared to rainwater (see table 6), concluding that the components selected decrease the pH of the rainwater.

Figure 19. Mean pH values from the runoff of vegetated systems (H. italicum (HI), C. album (CA), A. arenaria (AA) and polyculture (PL)), non-vegetated system (C) and rainwater (R) over eight rain events (S1-8). S1 corresponds to the first time that the substrate was percolated by rainwater and S8 corresponds to the last sampling date in June.

3.2.4.2 Electrical conductivity (EC)

Considering EC values along time (S1-8) (Fig.20) high variations were observed in vegetated and non-vegetated systems. In S1, the first rain event, the low values were probably associated to the high retention capacity of the newly installed substrate. S2 and S3 were a direct representation of ions concentration in the present substrate. After the third sampling date (S3) we can observe that the values of EC tended to stabilize between the sampling dates S4 and S7, however, in the sampling date S8, the values were higher. This was probably because S8 was characterized as strong storm event that lead to a substantial increase of ions content. We can conclude that the characteristics of the selected substrate and the characteristics of the rain event influence EC in water runoff.

No statistically significant differences ($p > 0.05$) were observed in EC between the tested systems (Control, Polyculture, *C. album* and *A. arenaria*), concluding that there was no influence by the different selected species as mono and polyculture when comparing to the non-vegetated Control. Statistical analysis showed that significant differences ($p < 0.05$) were obtained between the systems and rainwater. The mean EC were higher in the tested systems when compared to rainwater (see table 6). The initial values of COD in the systems (111 mg/L, 145 mg/L, 167 mg/L, 138 mg/L and 139 mg/L for Control, *C. album, H. italycum, A. arenaria* and polyculture, respectively) when compared with

rainwater (10 mg/L) indicated that the percentage of organic matter in the substrate selected was more than 10 times higher when compared to rainwater which in turn contributed to the elevated initial values of EC. We can therefore conclude that the components selected significantly increase ions concentrations in rainwater, being consistent with previous studies (e.g. Beecham and Razzaghmanesh, 2015; Buccola and Spolek, 2011).

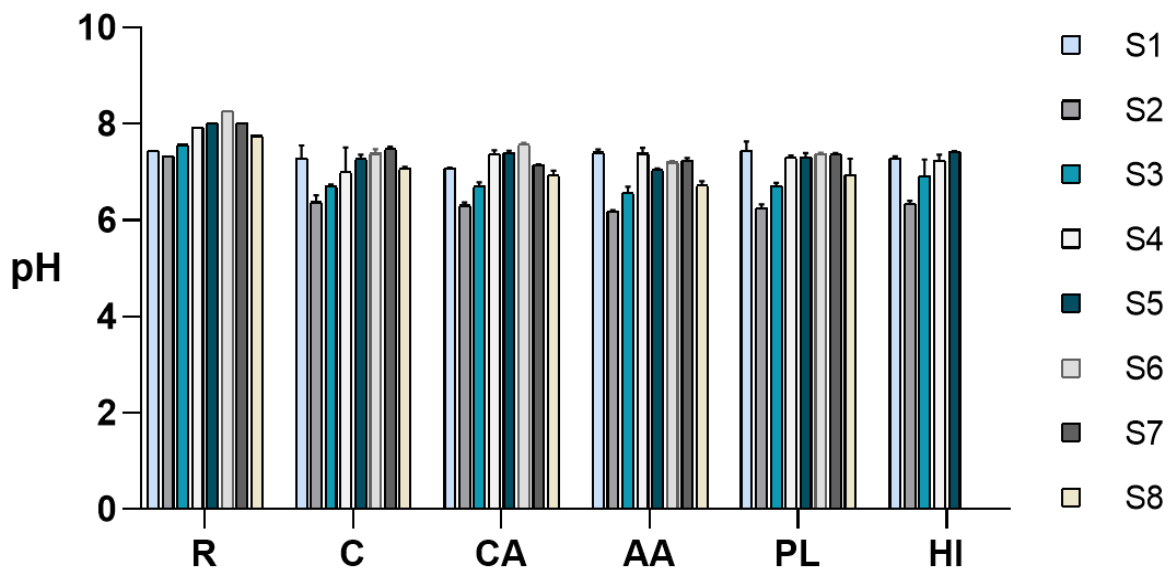Figure 20. Mean electrical conductivity (EC) values from the runoff of vegetated systems (H. italicum (HI), C. album (CA), A. arenaria (AA) and polyculture (PL)), non-vegetated system (C) and rainwater (R) over eight rain events (S1-8). S1 corresponds to the first time that the substrate was percolated by rainwater and S8 corresponds to the last sampling date in June.

3.2.4.3 Ammonium

Considering ammonium concentrations along time (Fig. 21) high variations were observed in vegetated and non-vegetated systems. It was observed a significant decrease in ammonium concentrations after the third sampling date S3, however, with a variable tendency. This decrease may be associated to the occurrence of leaching of ammonium in excess (in samplings S1-S3) that was not adsorbed by negatively charged components of the substrate and/or by the occurrence of nitrification (Mason *et al.*, 1999).

Statistical analysis showed that there were no significant differences ($p > 0.05$) between the tested systems and between the tested systems and the rainwater, concluding that the components selected didn't have a substantial influence on rainwater ammonium concentration. This is consistent with previous studies e.g. Vijayaraghavan *et al.* (2012),

where there were no observations of the systems working as a source or sink of ammonium.

Figure 21. Mean ammonium values from the runoff of vegetated systems (H. italicum (HI), C. album (CA), A. arenaria (AA) and polyculture (PL)), non-vegetated system (C) and rainwater (R) over eight rain events (S1-8). S1 corresponds to the first time that the substrate was percolated by rainwater and S8 corresponds to the last sampling date in June.

3.2.4.4 Nitrates

Considering nitrates concentrations along time (Fig. 22) a variable tendency was observed in vegetated and non-vegetated systems. This may be related not only with rainwater composition, rain event and substrate characteristics (Teemusk and Mander, 2007; Li and Babcock, 2014) but also with seasonally variations on N processing rates, which are influenced by temperature (S7 and S8 in figure 22 are from the hottest registered months of the experimental period). Further studies considering seasonality would possibly help to explain the big range of values obtained in the systems, since it's known that nitrates concentrations tend to increase with temperature (Buffam *et al.*, 2016).

Statistical analysis showed that there were no significant differences ($p > 0.05$) between the tested systems and between the tested systems and rainwater. Although the mean nitrates concentrations were slightly higher in the tested systems when compared to rainwater (see table 6), we conclude that the components selected did not have a considerable influence on rainwater nitrates concentration during the experimental time.

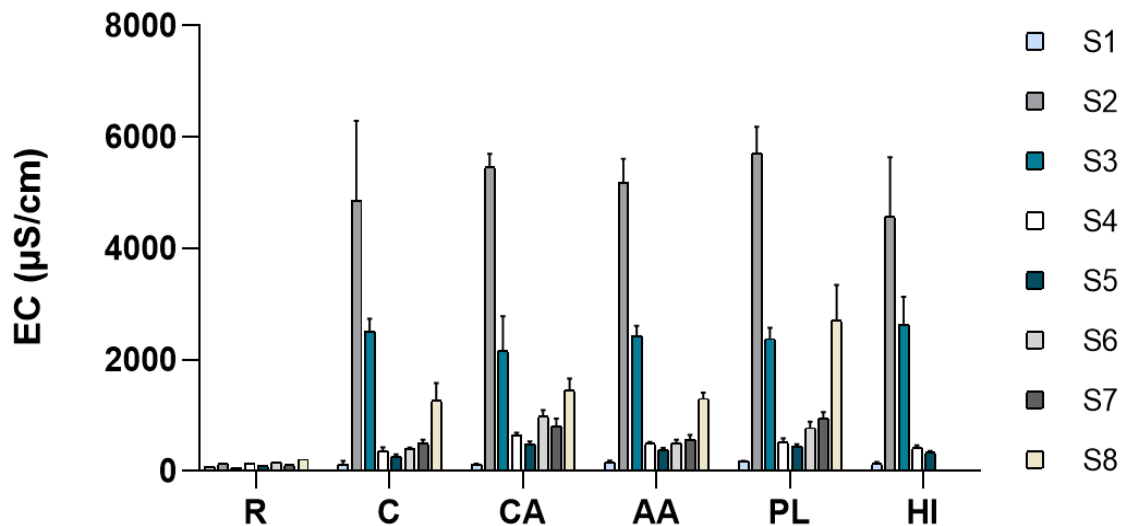*Figure 22. Mean nitrates values from the runoff of vegetated systems (H. italicum (HI), C. album (CA), A. arenaria (AA) and polyculture (PL)), non-vegetated system (C) and rainwater (R) over eight rain events (S1-8). S1 corresponds to the first time that the substrate was percolated by rainwater and S8 corresponds to the last sampling date in June.*

3.2.4.5 Phosphates

Considering phosphates concentrations along time (Fig. 23), a decreasing tendency was observed in vegetated and non-vegetated systems from S5 until S7. The increase of phosphates concentration in sampling date 8 may be related with an increase of mineralization rates by microorganisms of substrate organic matter due to higher temperatures and/or to the heavy storm event (Buffam *et al.*, 2016; Teemusk and Mander, 2007). Statistical analysis showed that there were significant differences ($p < 0.05$) among the systems and between rainwater and all the tested systems.

Statistically significant differences were observed between the tested systems ($p < 0.05$), of *C. album* and the systems of *A. arenaria* and Polyculture, and between the systems of Control and systems of *A. arenaria* and Polyculture. No significant differences were observed between Control and *C. album* probably due to the low survival capacity of the plant. Since the values between *A. arenaria* and Polyculture were similar, and those were the systems with better plant survival capacity, we consider that the lower values (see table 6) were probably related with plant uptake. High values seen in *H. italicum* (see table 6) were possibly related with increased organic matter resultant from plant litter which may have led to higher concentrations of phosphates by mineralization (Foth, 1991). Additionally, statistical analysis showed that there were significant differences (p

< 0.05) between rainwater and all the tested systems. Mean phosphates concentrations were higher in the tested systems when compared to rainwater (see Table 6), concluding that the components of the selected substrate significantly increase those ions concentrations in rainwater.
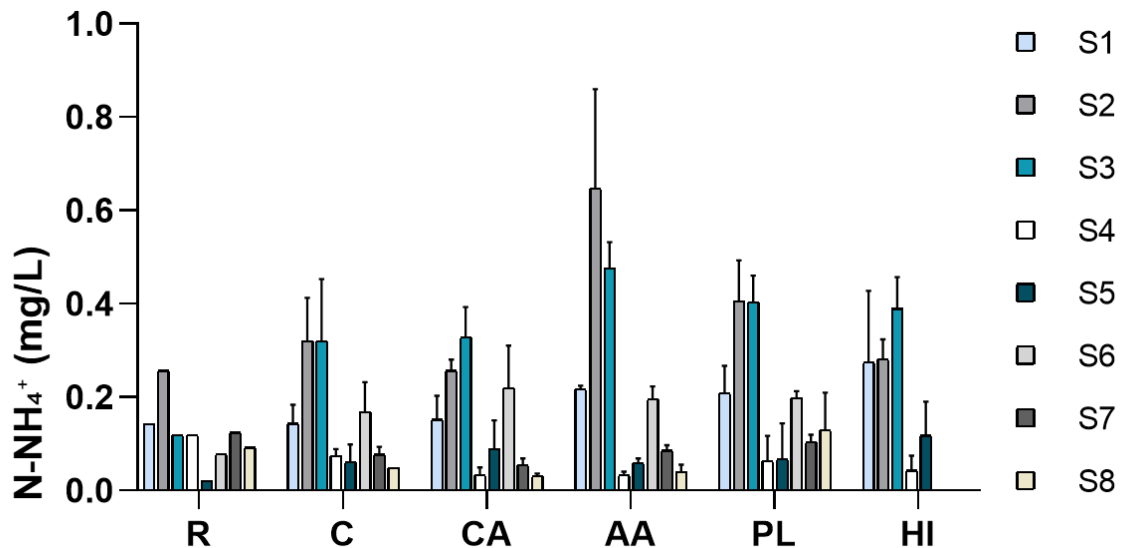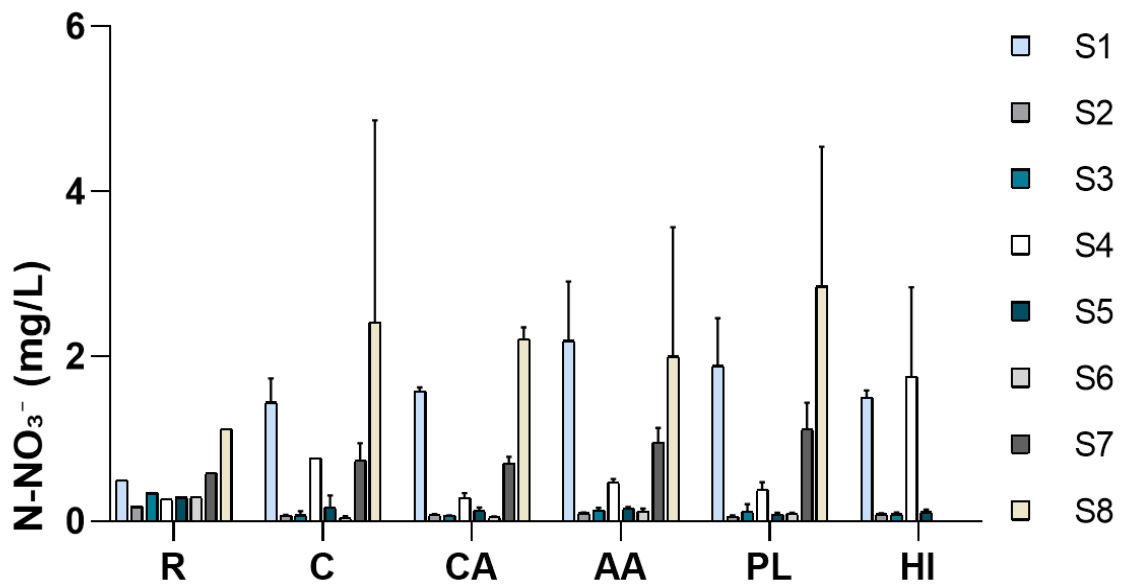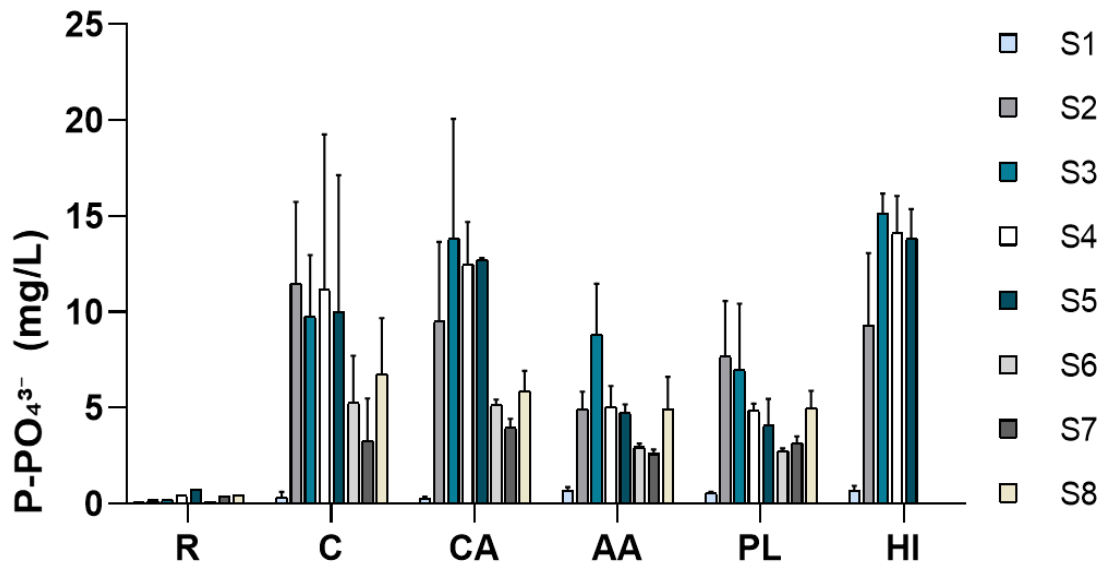

Figure 23. Mean phosphates values from the runoff of vegetated systems (H. italicum (HI), C. album (CA), A. arenaria (AA) and polyculture (PL)), non-vegetated system (C) and rainwater (R) over eight rain events (S1-8). S1 corresponds to the first time that the substrate was percolated by rainwater and S8 corresponds to the last sampling date in June.

Table 6. Water quality indicators of different water samples from the studied green roof systems and rainwater (N=8). Means, with minimum and maximum values, followed by letters representing the statistical analysis. Different letters mean statically significant differences (p<0.05).

Systems	Water runoff quality parameters,				
	pH	EC (µS/cm)	$N-NH_4^+$ (mg/L)	$N-NO_3^-$ (mg/L)	$P-PO_4^{3-}$ (mg/L)
Control	7.1 (6.3 – 7.6) a	1287 (77- 5740) a	0.16 (0.03 - 0.46) a	0.65 (0.02 - 4.14) a	7.28 (0.19 - 20.30) a
C. album	7.1 (6.2 – 7.6) a	1577 (98 - 5720) a	0.15 (0.02 - 0.37) a	0.69 (0.04 - 2.35) a	7.99 (0.20 - 17.50) a
A. arenaria	7.0 (6.2 – 7.5) a	1383 (138 - 5530) a	0.23 (0.03 - 0.87) a	0.71 (0.08 - 3.11) a	4.31 (0.49 - 11.80) b
Polyculture	7.1 (6.2 - 7.7) a	1708 (168 - 6210) a	0.20 (0.01 - 0.50) a	0.82 (0.05 - 4.47) a	4.46 (0.49 - 11.0) b
*H. italicum**	7.1 (6.4 – 7.4)	1724 (114 - 5800)	0.22 (0.02 - 0.47)	0.65 (0.06 - 2.70)	11.33 (0.54 - 16.30)
Rainwater	7.8 (7.3 – 8.3) b	121 (52 - 211) b	0.12 (0.02 - 0.26) a	0.45 (0.18 - 1.12) a	0.33 (0.10 - 0.75) c

*Data of H. italicum comprises only S1-5 sampling dates (N=5)

This seven-month experiment allowed us to conclude that the treatments in analysis presented variations between them and between sampling dates (S1-8). No statistically significant differences were observed in water runoff nutrients content between rainwater and the systems except in case of phosphates. In accordance with other studies (Berndtsson *et al*., 2006, Buffam *et al*., 2016 and Gregoire and Clausen, 2011) we observed that water runoff from both vegetated and non-vegetated systems contain higher levels of leached phosphates when comparing to rainwater, which in turn could impact downstream water in the initial stages after implementation. However, we observed lower phosphates values (by 40%) in systems with well-developed vegetation when comparing with the non-vegetated (control), probably as a result of its uptake. This is consistent with previous studies (e.g. Beecham and Razzaghmanesh, 2015), highlining the potential of vegetation influence on pollutants removal.

3.2.5 Temperature variations of green roof systems and conventional roof

As observed in the graph of figure 24, considerable differences were observed between the sensor established in the vicinity of the experiment (Roof) and sensors established under the substrates of the systems (Control, *C.album, A.arenaria* and Polyculture).
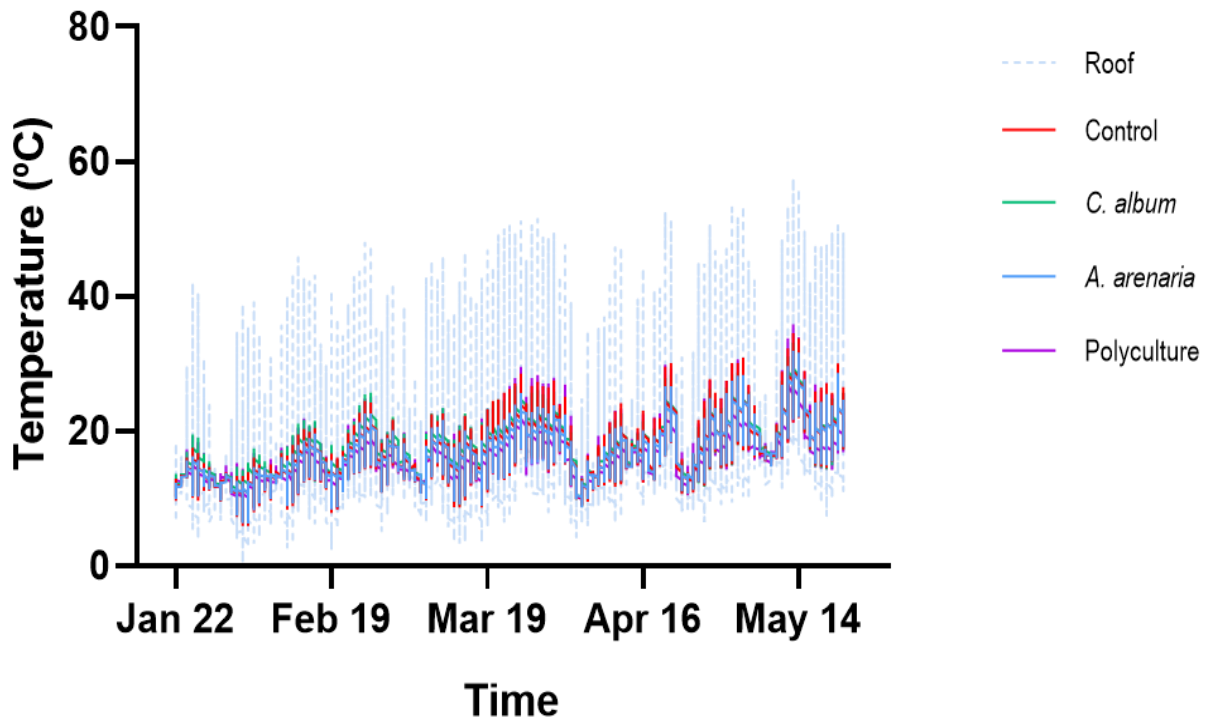
Figure 24. Temperature variations during the four-month experiment registered by sensors established under the substrate layers (of the control, C. album, A. arenaria and polyculture) and a sensor established in the vicinity of the experiment (roof).

Maximum and minimum temperatures observed in the roof, presented values of larger range than the systems in study. The maximum temperature values in all data registered in May and the minimum values in all data registered in February, were registered by the roof. At a minimum temperature of 0.34ºC registered in the roof, the registered temperature in green roofs systems was 5.88ºC for the control, 6.39ºC for *C. album*, 6.23ºC for *A. arenaria* and 6.23ºC for Polyculture. This shows that the green roof systems with all the selected components[11] can counteract between 5 to 6 ºC the decrease of temperature at the roof surface. At the maximum temperature of 57.48ºC registered in the roof, the registered temperature in green roofs systems was 34.54ºC for the control,

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[11] The drainage layer (ICB) is characterized by a thermal resistance of 1.96 m2ºC/W under a 100mm vegetated substrate

33.25 ⁰C for *C. album*, 31.94 ⁰C for *A. arenaria* and 35.82 ⁰C for Polyculture. This shows that the green roof systems can counteract between 22 to 25 ⁰C the increase of temperature at the roof surface.

Just as the results of Bevilacqua *and collaborators* (2015) our results indicate that the presence of vegetation cover had low impact on temperature stabilization. This is probably associated to the low plant density. Hereupon, the set of substrate, filter and drainage layer (ICB) were considered the main factors influencing temperature stabilization. Considering the values of the Roof when comparing with green roof systems, our results are in accordance with others (e.g. Teemusk and Mander, 2009; Bevilacqua *et al.*, 2016) showing that green roofs are capable to stabilize temperature oscillations better than regular roofs.

3.3 Performance comparison between two substrates with the same set of plants

3.3.1 Plant establishment

Considering the values of physiological parameters (ETR, α and Fv/Fm) of the selected plants (*Calystegia soldanella, Euphorbia paralias* and *Medicago marina)* no statistically significant differences ($p > 0.05$) were observed between the plant species over different substrates in study. All plants showed Fv/Fm values below the theoretical optimum of 0.83 measured for various plant species (Maxwell and Johnson, 2000), indicating that the plants were growing under some stress. However, all plants survived during the experimental period in both substrates, showing the potential to their use in green roofs. The lack of previous studies with this plant species in green roofs highlight the need of further studies to elucidate the suitability of using them.

3.3.2 Substrate characterization

Comparing the characteristics of both substrates the results showed that, on the contrary to the acidic nature of the commercial substrate, the experimental substrate had a neutral nature. It had also lower EC values, organic matter, organic carbon and potassium oxide content. Besides, regarding macronutrients content, were observed lower values of nitrogen and similar values of phosphorous pentoxide and C:N ration. Table 7 summarizes the results of the selected substrates, considering parameter and method of analysis

Table 7. Summary of experimental and commercial substrate characteristics.

Parameter	Method	Units	Experimental substrate	Commercial substrate
pH (H$_2$O)	ISO 10390:2005 / Potentiometry	pH units	7.55 ± 0.01	5.84 ± 0.01
pH (CaCl$_2$)	ISO 10390:2005 / Potentiometry	pH units	6.91 ± 0.01	5.56 ± 0.01
EC	ISO 11265:1994 / Conductometry	µS/cm	456 ± 2	1617 ± 2
Organic matter	Calculus (M.O. = Corg x 1,724)	%	11.34 ± 0.01	46.13 ± 0.01
Organic carbon	prNE 15936:2009 / Conductometry	%	6.58 ± 0.04	26.76 ± 0.04
Total nitrogen	NE 13654-2:2001 / Conductometry	%	0.13 ± 0.02	0.57 ± 0.02
Ratio C : N	Calculus	%	49.0	47.2
Phosphorous pentoxide (P$_2$O$_5$)	ISO 22036:2008 / Mehlich extraction 3	mg/kg	136.2 ± 0.4	139.3 ± 0.4
Potassium oxide (K$_2$O)	ISO 22036:2008 / Mehlich extraction 3	mg/kg	417.6 ± 1.3	1595.2 ± 1.3

3.3.3 Water quality

This section is reserved to evaluate if there were significant differences between the tested treatments (experimental substrate (E) and commercial substrate (CM) with the same set of plants) and what is the influence of those green roof systems in rainwater that percolates those systems.

3.3.3.1 pH

Considering mean pH values (Fig. 25), the results show that there were statistically significant differences between the experimental and commercial substrates. However, when comparing with rainwater, no significant differences were observed with both substrates. The mean pH value of the commercial substrate demonstrated to be slightly lower than the rainwater and the experimental substrate (see table 8). This in turn, may be related to the substrate characteristics, since the commercial substrate has a more acidic nature.

Figure 25. Mean pH values from the runoff of experimental (E) and commercial (CM) substrates with the same set of plants (C. soldanella, M. marina and E. paralias) and rainwater (R) over three rain events. Different symbols (■○) denote statistically significant differences (p < 0.05) (Ordinary one-way ANOVA).

3.3.3.2 Electrical conductivity

Considering mean electrical conductivity EC) values (Fig. 26), the results show that there were statistically significant differences between the tested systems (the experimental and commercial substrates) and rainwater. The mean EC were higher in the tested systems when comparing to rainwater, concluding that the components selected significantly increase ions concentrations of the rainwater. This is consistent with previous studies (e.g. Beecham and Razzaghmanesh, 2015; Buccola and Spolek, 2011). The higher values of COD (164 mg/L in experimental substrate and 142 mg/L in commercial substrate) when compared to rainwater indicate that the percentage of organic matter in the substrates selected were higher when compared to rainwater, which in turn contributed to the higher values of EC. No statistically significant differences were observed between the tested systems, concluding that there was no significant influence by the different substrates.

Figure 26. Mean of electrical conductivity values from the runoff of experimental (E) and commercial (CM) substrates with the same set of plants (C. soldanella, M. marina and E. paralias) and rainwater (R) over three rain events. Different symbols (■○) denote statistically significant differences (p < 0.05) (Ordinary one-way ANOVA).

3.3.3.3 Ammonium

Considering mean ammonium values (Fig. 27), the results show that there were no statistically significant differences between the treatments in analysis. However, on the contrary to the experimental substrate, the commercial substrate showed higher concentrations of ammonium in water runoff, concluding that the substrate components promote a slight increase of ions concentrations in rainwater (Fig. 27; Table 8). The lower values of the experimental substrate may be associated with the lower organic matter percentage and composition (as pine bark and peat, present in the commercial substrate, have low capacity to retain $N-NH_4^+$) and high composition in clay (mineral fraction), which in turn present higher cation exchange capacity (Bunt, 1988).
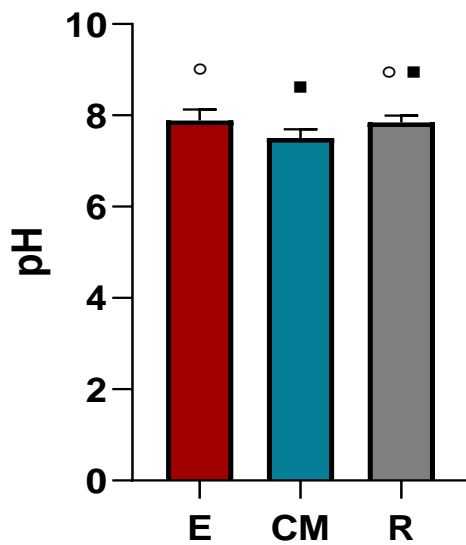
Figure 27. Mean ammonium values from the runoff of experimental (E) and commercial (CM) substrates with the same set of plants (C. soldanella, M. marina and E. paralias) and rainwater (R) over three rain events. Different symbols (▪○) denote statistically significant differences (p < 0.05) (Ordinary one-way ANOVA).

3.3.3.4 Nitrates

Considering mean nitrates values from the experimental and commercial substrates (Fig. 28), the results showed that there were no statistically significant differences between them. However, when comparing with rainwater, significant differences were observed with both systems. Since higher concentrations of nitrates were find in the tested systems, we conclude that the components selected increase ions concentrations on the rainwater.
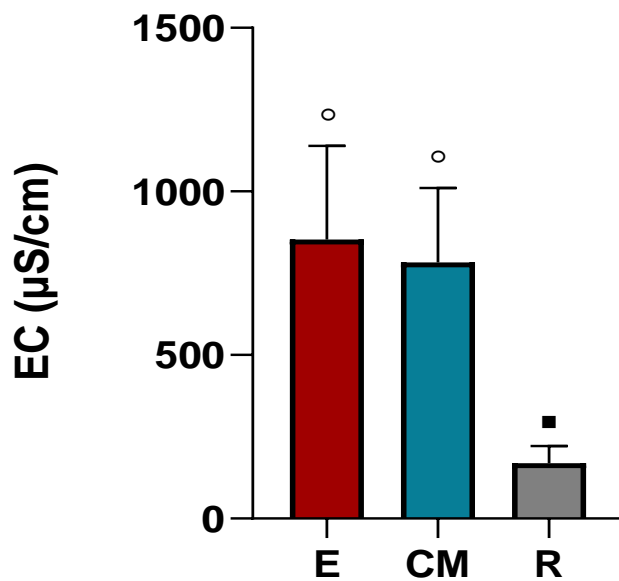
Figure 28. Mean nitrates values from the runoff of experimental (E) and commercial (CM) substrates with the same set of plants (C. soldanella, M. marina and E. paralias) and rainwater (R) over three rain events. Different symbols (▪○) denote statistically significant differences (p < 0.05) (Ordinary one-way ANOVA).

3.3.3.5 Phosphates

Considering mean phosphate values (Fig. 29), the results showed that there were significant differences between all the treatments in analysis. Both substrates showed higher values of phosphates when comparing with rainwater. The experimental substrate showed the highest concentration of phosphates, possibly related with the high percentage of the finest fraction (sand) in the substrate with less ion affinity (Lehmann and Schroth, 2003) concluding that the components lead to an increase of ions concentrations on the rainwater that percolates de systems. The lower values of the commercial substrate may be associated with its higher retention capacity, which in turn, is associated to a higher organic matter content (Lambrinos, J., 2015).
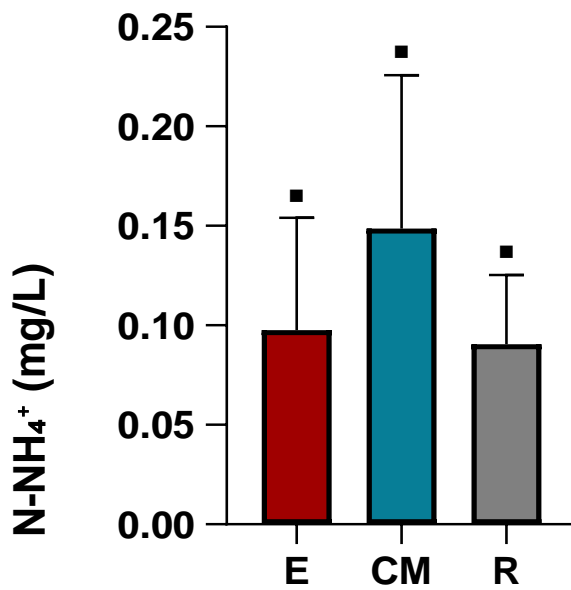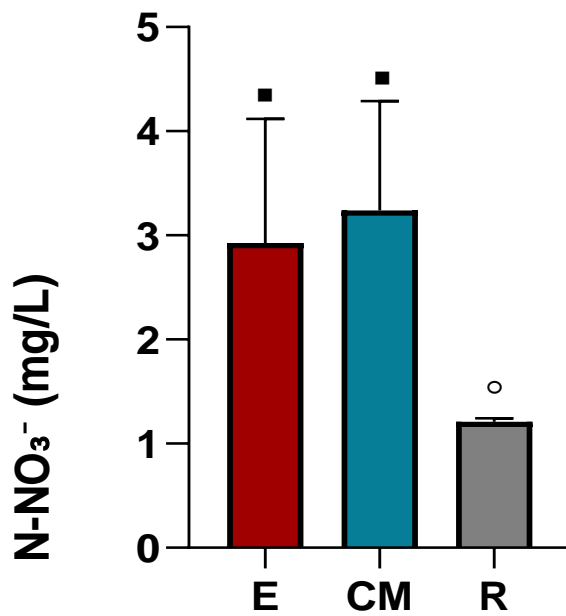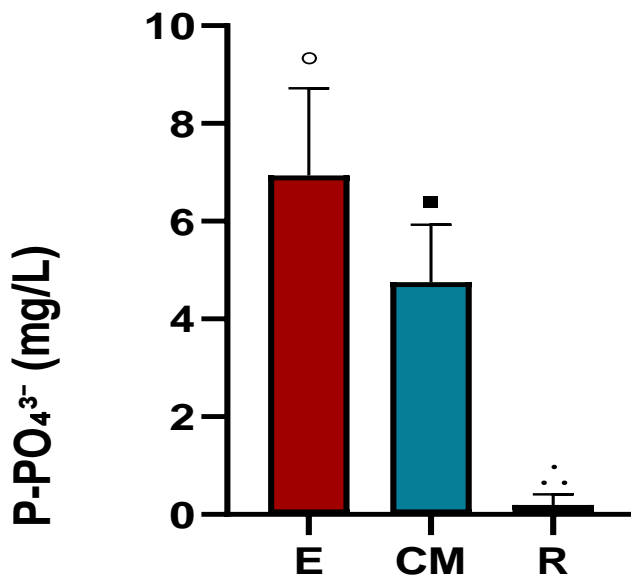
Figure 29. Mean phosphates values from the runoff of experimental (E) and commercial (CM) substrates with the same set of plants (C. soldanella, M. marina and E. paralias) and rainwater (R) over three rain events. Different symbols denote statistically significant differences (p < 0.05) (Ordinary one-way ANOVA).

The five-month experiment allowed us to conclude that there were distinct results between treatments associated to the nature of substrates. When compared to rainwater, the experimental substrate showed similar values of pH and ammonium (see table 8). On the other hand, the experimental substrate presented significantly higher values of phosphates (30% higher) when compared to the commercial substrate (Table 8). Comparing with rainwater, the commercial substrate had lower pH values, but presented higher values in all other parameters of analysis (EC, NH_4^+, NO_3^- and PO_4^{3-}). Hereupon, in accordance with other studies (e.g. Vijayaraghavan *et al.*, 2012; Aitkenhead-Peterson *et al.*, 2011) we can conclude that both systems acted as a source of nutrients to rainwater (namely NO_3^- and PO_4^{3-}).

Altering substrate composition e.g. organic matter type and amount could therefore lead to better quality of water runoff (Harper *et al.*, 2015).

Table 8. Water quality indicators of different water samples from the studied green roof systems (experimental substrate and commercial substrate) and rainwater (N=3). Means, with minimum and maximum values, followed by letters representing the statistical analysis. Different letters mean statically significant differences (p<0.05).

Systems	Water runoff quality parameters				
	pH	EC (µS/cm)	NH_4^+ (mg/L)	NO_3^- (mg/L)	PO_4^{3-} (mg/L)
Experimental substrate	7.9 (7.7 - 8.2) a	853 (516 - 1387) a	0.10 (0.03 - 0.19) a	2.2 (0.1 – 4.6) a	6.9 (4.5 – 9.5) a
Commercial substrate	7.5 (7.3 – 7.8) b	782 (529 - 1068) a	0.15 (0.02 - 0.23) a	2.6 (0.2 – 4.5) a	4.8 (2.9 – 6.2) b
Rainwater	7.8 (7.8 - 8.0) a,b	169 (111 - 211) b	0.09 (0.06 - 0.12) a	0.8 (0.1 – 1.2) b	0.2 (0.1 - 0.4) c

3.3.4 Temperature variations of green roof systems and conventional roof

As observed in the graph of temperature variations (Fig. 30), significant differences were observed between the sensor established in the vicinity of the experiment (Roof) and sensors established under the commercial substrate with *Calystegia soldanella, Euphorbia paralias* and *Medicago marina*.
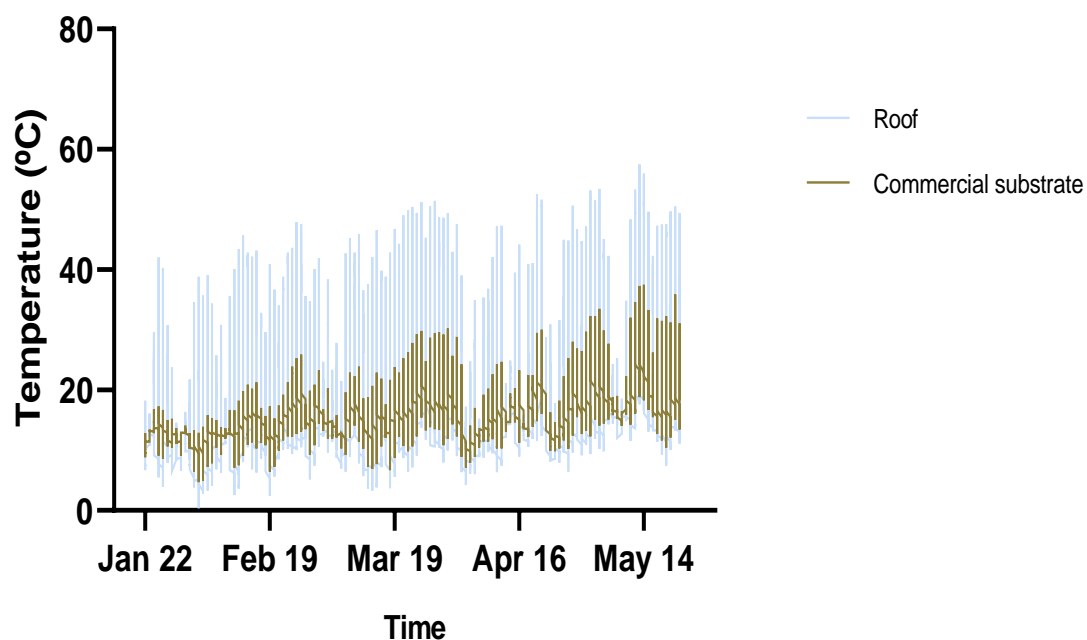
Figure 30. Temperature variations during the four-month experiment registered by sensors established under the substrate layer of the commercial substrate and a sensor established in the vicinity of the experiment.

Maximum and minimum temperatures observed in the roof, presented values of larger range than the commercial substrate. The maximum temperature values in all data registered in May and the minimum values in all data registered in February, were registered by the roof. At a minimum temperature of 0.34 ºC registered in the roof, the registered temperature in the commercial substrate was 4.73 ºC. This shows that the green roof system can counteract approximately 4 ºC the decrease of temperature at the roof surface. At the maximum temperature of 57.48 ºC registered in the roof, the registered temperature in the commercial substrate was 37.51 ºC. This shows that the green roof system can counteract approximately 20 ºC the increase of temperature at the roof surface. This is probably associated to the fact that the substrate store less heat than the roof material (Pérez *et al.*, 2015). In the graph, the experimental substrate is not indicated due to data loss, being only considered the sensor data from the roof and from the commercial substrate. Our results in accordance with the results of Teemusk and Mander (2009), indicating that the green roof system, with all the selected components[12], shows potential to assure good insulation characteristics.

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[12] The drainage layer (ICB) is characterized by a thermal resistance of 1.96 m2ºC/W under a 100mm vegetated substrate.

3.4 Green roof economics

An estimated value of the components per m³ was accomplished in order to reveal the estimated price of a green roof system with the components selected in our study case, namely: ICB, filter layer, substrate layer and vegetation layer. In Table 9 it's described the estimated price per m³ of the green roof system components, including the commercial substrate and in Table 10 it's described the estimated price per m³ of the green roof system components, including the experimental substrate. The final values may vary depending on the supplier and roof dimension.

Table 9. Estimated price per m³ of the green roof system composed by ICB, filter layer, commercial substrate and plants.

Component	Price (€)* / m³
ICB	42
Filter layer	2.38
Commercial substrate	140.12
Plants	~ 30**

*IVA not included
**Considering that we would plant 15 plants, with an approximate value of 2 €

Table 10. Estimated price per m³ of the green roof system composed by ICB, filter layer, experimental substrate and plants.

Component	Price (€)* / m³
ICB	42
Filter layer	2.38
Experimental substrate	54.29
Plants	~ 30**

*IVA not included
**Considering that we would plant 15 plants, with an approximate value of 2 €

Chapter IV

CONCLUSION AND FUTURE PERSPECTIVES

4. CONCLUSION AND FUTURE PERSPECTIVES

This multifunctional study provides important information to the design of Green Roofs under coastal conditions. Understanding how components selection influence the dynamics of a Green Roof is of great interest. Plants selected must survive in the selected substrate, and together, they can improve not only the aesthetic value of the roof but also contribute to temperature stabilization, nutrient cycling and water runoff quality. In experiment I, *Ammophila arenaria* in the selected commercial substrate, showed to be a good candidate to green roof systems under coastal conditions. The system presents great potential to improve temperature stabilization and possibly keep nutrient cycling in green roof substrates through their associated microbial communities. Consequently, the development of important microbial communities (e.g. nitrogen fixing bacteria) associated to plant rhizosphere may lead to a green roof free of maintenance in terms of fertilization. This study is among the first studies of substrate microbial characterization and analysis of potential beneficial bacteria to these systems. Studies of longer duration and higher plant density would help to observe clearer changes in microbial communities and explore their potential as this ecosystem mature and plants reproduce. Further studies considering growing season, substrate proprieties and/or irrigation needs of *Corema album* and *Helichrysum italicu*m would elucidate about the adequacy of using these plants. Regarding water quality evaluation, we conclude that all systems served as a source of phosphates to rainwater during the experimental time. Studies of longer duration would elucidate about the influence of seasonality and if their concentration in water runoff would tend to decrease over time. In experiment II, *Calystegia soldanella, Euphorbia paralias* and *Medicago marina* had similar photosynthetic capacity in both substrates in study. This shows that the lower organic matter percentage and higher mineral content of the experimental substrate was not enough to improve plant performance. Nevertheless, both presented to be suitable to face green roof conditions and support vegetation growth in coastal areas. Furthermore, our results indicate that the different substrate characteristics did not show any significant differences in water quality parameters since both acted as a source of nutrients (phosphates and nitrates) in water runoff.

In conclusion, although different experiments, with different work objectives, both systems demonstrated to be a source of macronutrients in water runoff. This underlines the need of additional studies to test materials with lower macronutrients content, but also capable to support plant survival. Furthermore, studies of longer duration would help

to understand the impact on water quality by plant uptake and how leachate nutrients concentrations change along time. Lower temperature variations were observed in all systems when comparing to the Roof. This could entail greater insultation characteristics to the roof when using these components and possibly lead to energy savings. The macrofauna characterization allowed to conclude that both experiments showed potential to function as habitat for various groups of organisms. Hereupon, with adequate components, green roofs may support plant and animal biodiversity conservation using endangered plants and attracting animals, increasing green areas in growing cities.

Lastly, with the intention of Green Roofs become a sustainable practice, all components must be taken in consideration. This would help to diminish the environmental impact and hence increase the durability of the system with minimum irrigation and maintenance. This approach would also support a strategy to use green roofs as nature-based solution for climate mitigation and adaptation. Thus, to achieve that purpose, it is necessary to use resistant plants to unfavorable conditions, the selection of a proper substrate to promote their growth and the use of components with low ecological footprint (e.g. products from industrial waste or recycled materials).

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Communications

Communications in National Scientific Symposiums

Silva, V., Arenas, F., Mucha, A. P., Palha, P. and Calheiros, C. S. C. "Green roofs implementation and assessment in coastal areas" in Encontro de Investigação Jovem da Universidade do Porto, 2019
Silva, V., Mucha, A. P., Arenas, F., Almeida, C. M., Palha, P., Calheiros, C. S. C. "Assessment of green roofs with different plant species in first sea line" in VII AEICBAS BIOMEDICAL CONGRESS, 2019.

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Silva, V., Mucha, A. P., Arenas, F., Almeida, C. M., Palha, P., Calheiros, C. S. C. "Green roofs implementation and assessment in coastal areas" in 3rd European Urban Green Infrastructure Conference, 2019.

ANNEXES

Annex I Filter layer of thermoset propylene data sheet

Figure 31. Filter layer of thermoset propylene data sheet

Annex II Commercial substrate data sheet

Substrato Landlab Intensivas

1. NATUREZA E QUALIDADE DO SUBSTRATO

O Substrato técnico Intensivas, Landlab – desenvolvido segundo a normativa FLL; constituído por componentes especiais com base mineral, que lhe conferem uma textura meia-grossa, capilaridade e drenagem elevadas e equilibradas. Este substrato caracteriza-se por apresentar uma elevada componente mineral, isento de parasitas, espécies infestantes e germes fito patogénicos e grande resistência estrutural.

2. COMPOSIÇÃO DO SUBSTRATO TÉCNICO LANDLAB INTENSIVAS

· Húmus de casca de pinho fermentado e certificado, granulometria 0-15mm

· Turfa loura selecionada, granulometria 0-40 mm

· Argila Expandida - granulometria 2/4mm

· Rocha vulcânica especial, granulometria 3-9mm

· pH corrigido para 5.5-6.5

· Densidade específica: 750-500kg/m humidade natural (50-60%)

· Densidade quando saturado: 650-700 kg/m^3

Figure 32. Commercial substrate data sheet

ANNEX III Geographic distribution of the selected plants of experiment I

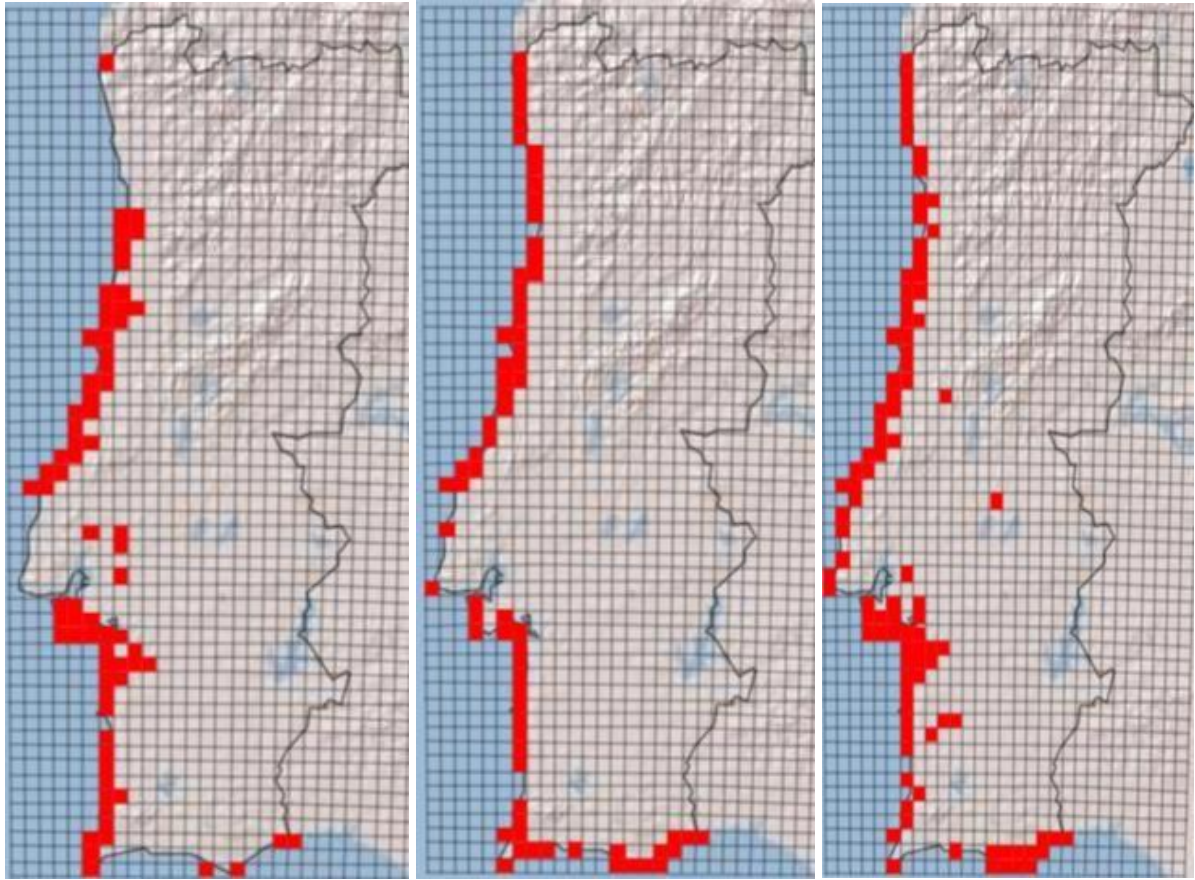*Figure 33. Continental Portugal distribution of the selected plants in experiment I. From the left to the right: Corema album, Ammophila arenaria and Helichrysum italicum (Flora-On: Flora de Portugal Interactiva, 2014).*

ARGEX® 0-2

DOC 04.01-001 REV00

Ficha Técnica

DESCRIÇÃO

A ARGEX® 0-2 é um agregado leve de argila que é expandida em forno rotativo a 1200ºC. É um produto natural, leve, resistente, incombustível, não inflamável e inalterável com o tempo.

CAMPO DE APLICAÇÃO

Argamassas e rebocos leves com bom comportamento térmico e de resistência ao fogo.
As propriedades mecânicas e físicas do agregado tornam-no indicado para utilização em elementos pré-fabricados com características especiais.

CARACTERÍSTICAS

ARGEX® 0-2	Valor declarado	Unidades
Classes granulométricas reais	0,25 – 2,0	mm
Densidade aparente seca (Baridade)	550	(±15%) kg/m³
Superfícies esmagadas e partidas	N.A.	(% massa)
Resistência ao esmagamento (± 10%)	7,7	MPa
Absorção de água	27,3	(% massa seca)
Resistência ao fogo	Incombustível Euro Classe A1	-

RECOMENDAÇÕES DE APLICAÇÃO

A ARGEX® pode ser utilizada como agregado leve de uma argamassa com dosagem estudada. Na execução de betonilhas ou betões, efetuar a mistura com apenas 2/3 da água prevista, juntando a restante no final, para prevenir que o excesso de água provoque a flutuação da ARGEX®.

MODO DE FORNECIMENTO

A ARGEX® 0-2 encontra-se disponível em embalagens de 1,5 m³ e de 3,0 m³. Também pode ser fornecida a granel com carga em fábrica a partir de silo para camião, ou camião cisterna.
O volume considerado é o volume aparente, não comprimido, na fábrica.

TRANSPORTE E ARMAZENAGEM

Durante o transporte e/ou a armazenagem podem ocorrer segregação de finos, alterações no teor de humidade e diminuição de volume aparente devido ao rearranjo do material, à semelhança de outros inertes tais como areia e brita.

HIGIENE E SEGURANÇA

Produto inerte, não representa perigo para a saúde nem para o ambiente. Em algumas aplicações podem ocorrer a formação de poeiras pelo que é conveniente a utilização de máscara de proteção.

ARGEX – Argila Expandida, S.A.
Zona Industrial, Azurveira, 3770-011 Bustos, Aveiro - Portugal
T (+351) 234 751 533   F (+351) 234 751 534   @ argex@argex.pt   W www.argex.pt

Figure 34. Expanded clay 0-2 data sheet

ARGEX® 2-4

DOC 04.01-002 REV00

Ficha Técnica

DESCRIÇÃO

A ARGEX® 2-4 é um agregado leve de argila que é expandida em forno rotativo a 1200ºC. É um produto natural, leve, resistente, incombustível, não inflamável e inalterável com o tempo.

CAMPO DE APLICAÇÃO

Betões leves com bom comportamento térmico e acústico e betões estruturais leves para obras especiais e de reabilitação. As propriedades mecânicas e físicas do agregado tornam-no indicado para utilização em elementos pré-fabricados com características térmicas e acústicas, tais como blocos e abobadilhas.

CARACTERÍSTICAS

ARGEX® 2-4	Valor declarado	Unidades
Classes granulométricas reais	4,0 – 8,0	mm
Densidade aparente seca (Baridade)	358	(±15%) kg/m³
Superfícies esmagadas e partidas	N.A.	(% massa)
Resistência ao esmagamento (± 10%)	4,8	MPa
Condutibilidade térmica	0,11	(W/m.ºC)
Absorção de água	26,2	(% massa seca)
Resistência ao fogo	Incombustível Euro Classe A1	-

RECOMENDAÇÕES DE APLICAÇÃO

A ARGEX® pode ser utilizada solta, regada com aguada de cimento ou como agregado leve de um betão com dosagem estudada. Na execução de betonilhas ou betões, efetuar a mistura com apenas 2/3 da água prevista, juntando a restante no final, para prevenir que o excesso de água provoque a flutuação da ARGEX®.

MODO DE FORNECIMENTO

A ARGEX® 2-4 encontra-se disponível em sacos de 50 litros e embalagens de 1,5 m³ e de 3,0 m³. Também pode ser fornecida a granel com carga em fábrica a partir de silo para camião, ou camião cisterna. *O volume considerado é o volume aparente, não comprimido, na fábrica.*

TRANSPORTE E ARMAZENAGEM

Durante o transporte e/ou a armazenagem podem ocorrer segregação de finos, alterações no teor de humidade e diminuição de volume aparente devido ao rearranjo do material, à semelhança de outros inertes tais como areia e brita.

HIGIENE E SEGURANÇA

Produto inerte, não representa perigo para a saúde nem para o ambiente. Em algumas aplicações podem ocorrer a formação de poeiras pelo que é conveniente a utilização de máscara de proteção.

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Figure 35. Expanded clay 2-4 data sheet.

Table 11. Sand characteristics data sheet

Norma harmonizada	EN 13139:2002	EN 13139:2002/AC:2004		
Características	Desempenho	Granulometria típica		
Dimensão Nominal - Tamanho das partículas	0/1			
Massa Volúmica (Mg/m³)	ρ_a = 2,56 - 2,66 ρ_{rd} = 2,54 - 2,64 ρ_{ssd} = 2,55 - 2,65	Abertura (mm)	(%)	Tolerância (%)
Absorção de agua	< 0,7%			
Teor de finos	Categoria 1	2	100	100
Teor de cloretos	< 0,01%	1	99	95-100
Teor de sulfatos solúveis em ácido	AS $_{0,2}$	0,250	15	0-40
Teor de enxofre total	< 0,1%	0,063	0,0	0,0-5,0
Teor de húmus	Mais claro que padrão			

Notas: Resultados que não constam, não foram realizados ou não solicitados.
A origem dos valores dos ensaios são da responsabilidade do produtor da matéria-prima.
Mais informações disponível na Ficha de Dados de Segurança

Table 12. Universal substrate characteristics (https://www.aki.pt/Substrato-Universal-45L-ECO-Grow-P24586.aspx#0)

Composition	Forest Waste Humus and Blonde Peat
pH	6
% organic matter	70

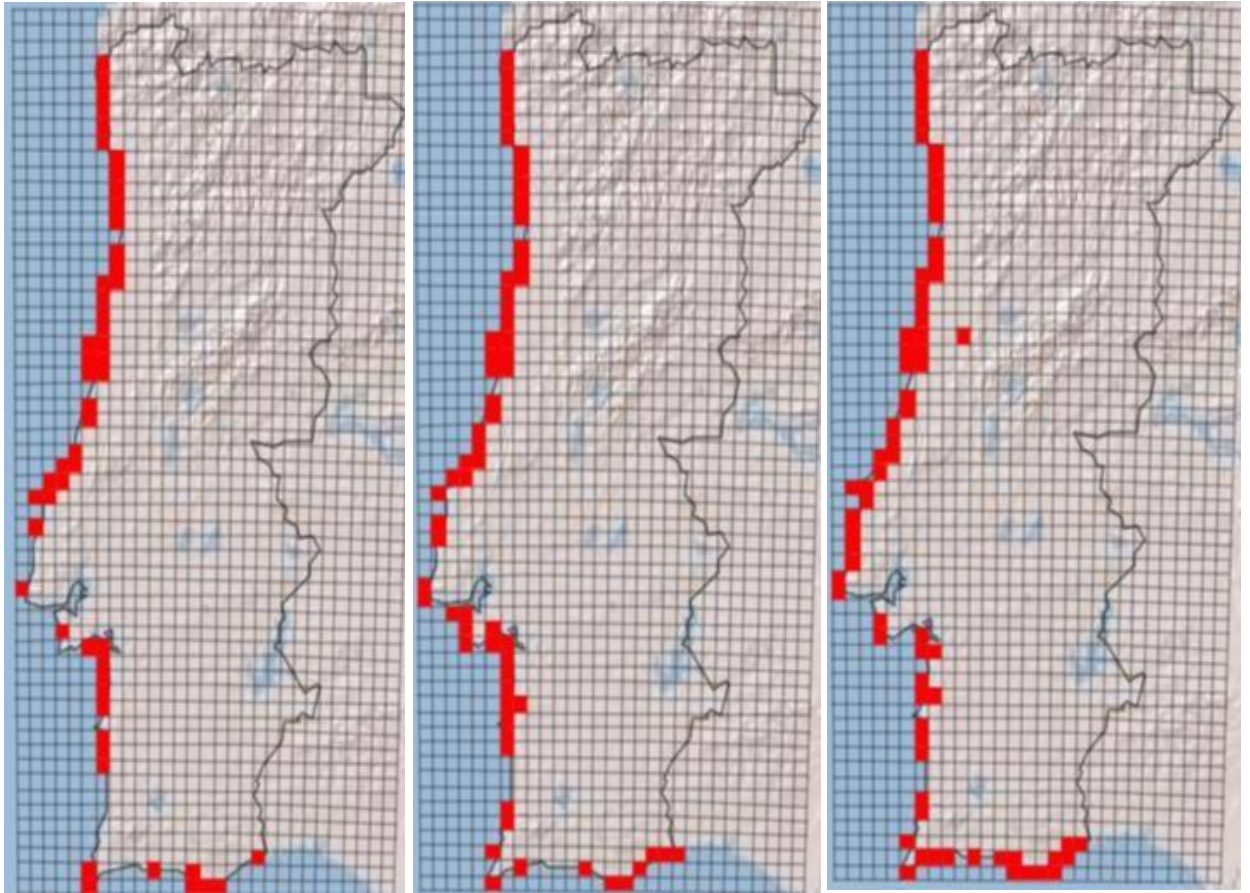**ANNEX V Geographic distribution of the selected plants of experiment II**

Figure 36. Continental Portugal distribution of the selected plants in experiment II. From the left to the right: Calystegia soldanella, Euphorbia paralias and Medicago marina (Flora-On: Flora de Portugal Interactiva, 2014).